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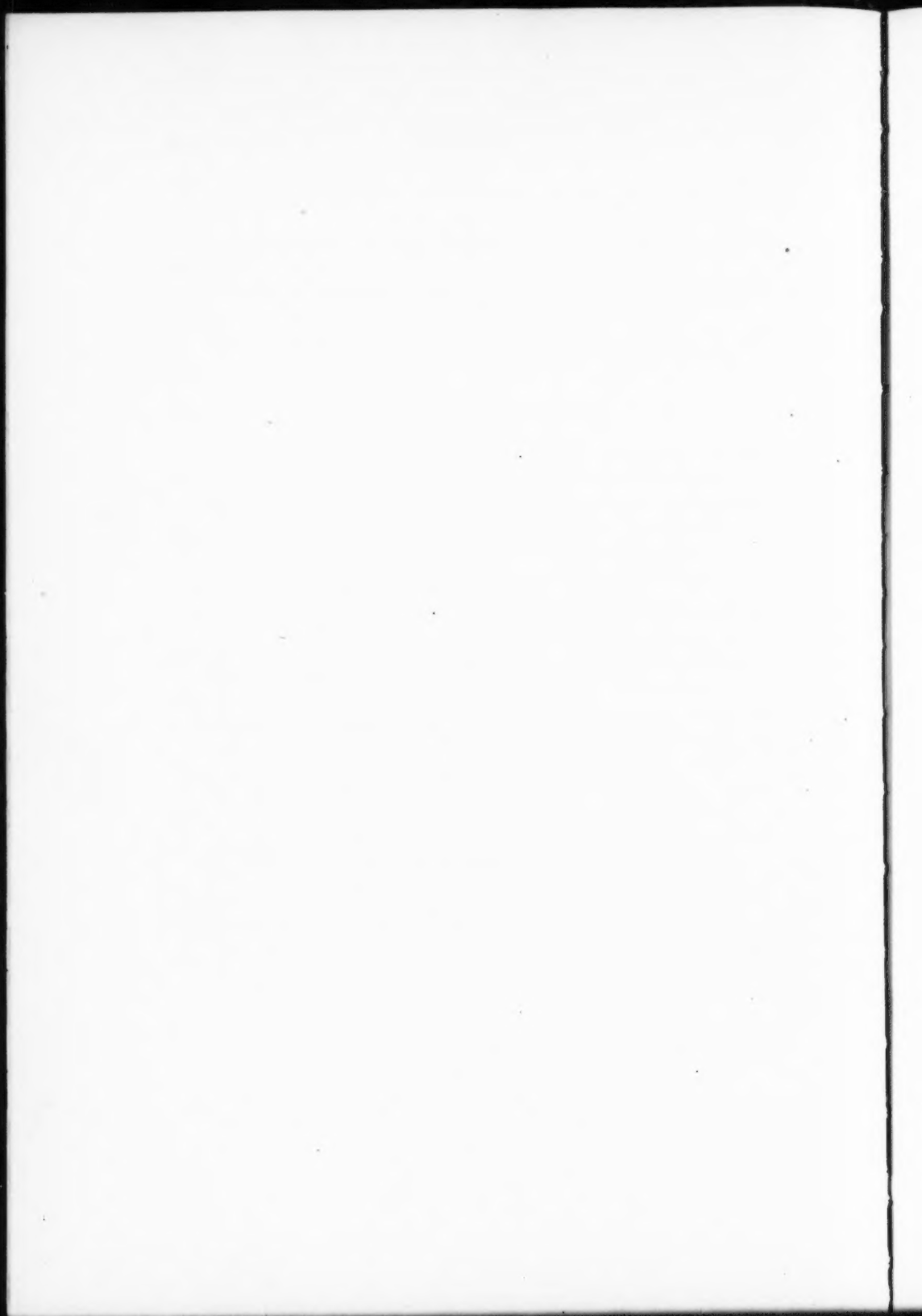
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DECREASE BY ANÆSTHETICS IN THE RATE OF TOXIC
ACTION OF PURE ISOTONIC SALT SOLUTIONS ON
UNFERTILIZED STARFISH AND SEA-URCHIN EGGS.

By RALPH S. LILLIE.

[From the Marine Biological Laboratory, Woods Hole, and the Physiological Laboratory
Department of Zoology, University of Pennsylvania.]

INTRODUCTORY.

IN a recent paper¹ I have shown that various anæsthetics (ether, chloroform, alcohol, chloretone, chloral hydrate, benzol, toluol, xylol), in concentrations identical with those which produce typical anæsthesia, prevent or retard the disintegrative or toxic action of pure isotonic solutions of sodium chloride on the cilia and muscles of *Arenicola* larvæ. This antitoxic action is associated with a decrease or prevention of the marked and rapid increase in permeability which the pure salt solution acting by itself normally produces. The protective or anticytolytic action of the anæsthetic is thus to be referred to its action on the plasma membrane, — this structure being so modified that the salt solution is no longer able to induce the same marked and rapid increase of permeability as before. Anæsthetics thus produce an effect similar — though less favorable — to that of salts like calcium or magnesium chloride, which also prevent rapid increase of permeability and at the same time diminish the toxicity of the pure salt solution. Further proof is thus afforded that certain forms of

¹ This Journal, 1912, xxix, p. 372.

antitoxic action depend primarily on a modification of the impermeable surface layer or plasma membrane of cells, and consist in rendering this structure more resistant to conditions that in the absence of the antitoxic agent increase permeability to an abnormal and injurious degree.²

In the present paper I shall describe the results of similar experiments with the unfertilized eggs of starfish and sea-urchins. In each series of these experiments the eggs were exposed for definite periods to (1) the pure isotonic solution of the salt used, and (2) to a series of solutions containing in addition to the salt various anæsthetics in known concentrations. In the majority of series calcium chloride and potassium cyanide were also used for comparison. The relative toxicity of the solutions was estimated (1) by observing the rate of cytolytic action upon the eggs while in the solution; this action is shown in *Asterias* eggs by a gradual darkening or coagulation of the protoplasm, and in *Arbacia* eggs by the diffusion of pigment into the solution; and (2) by fertilizing the eggs in sea water after a definite stay in the solution and determining the proportion that develop to a larval stage; the proportion of such eggs as compared with the proportion developing from normal untreated eggs is an accurate index of the degree of injury undergone.

The results of these experiments have shown that the addition of anæsthetics may have a distinct and often pronounced effect in retarding the toxic action of pure isotonic solutions of sodium and potassium salts. This protective or antitoxic effect is most readily demonstrated in those solutions where the cytolytic action is sufficiently rapid to kill all of the eggs in a comparatively brief time, *i. e.*, within one to two hours. Sodium chloride solution has this action on normal mature starfish eggs; in the presence of anæsthetics the life of the egg in the solution is decidedly prolonged (see below, page 7). On

² The reasons why mere increase of surface permeability is injurious or destructive to the cell are discussed in my earlier paper on the general biological significance of changes of permeability, in *Biological bulletin*, 1909, xvii, pp. 192 ff. The conclusion is there reached that "in general toxic action must be regarded as depending in many cases primarily on an alteration, particularly an *increase*, in the normal permeability of the cells." Further evidence that antitoxic action consists in a prevention of this increase is presented in my papers on permeability changes in relation to stimulation and the initiation of cell division, in this *Journal*, 1909, xxiv, pp. 23 ff., 486 ff., and 1910, xxvi, pp. 117 ff.

Arbacia eggs the action of sodium chloride is much more gradual, and in most of the experiments with these eggs the more rapidly acting salts, iodide, sulphocyanate, and nitrate — of both sodium and potassium — were used. Pure isotonic solutions of all these salts, with the exception of KNO_3 , kill unfertilized Arbacia eggs within one and a half hours or less, sodium being distinctly more toxic than potassium; in all of these solutions various anæsthetics in appropriate concentrations decrease the rate of toxic action, *i. e.*, exert a definite antitoxic influence, as the experiments described below will illustrate. In the case of more gradually acting salt solutions, such as sodium chloride, which requires some hours to kill all of these eggs, anæsthetics have little or no antitoxic effect.

The rate of action of the salt solution is thus an important factor in determining the possibility and the degree of counteraction through anæsthetics. Apparently these substances check a rapid action, but have little effect on one more gradual. It seems probable that anæsthetics protect unfertilized Arbacia eggs against solutions of sodium iodide or potassium sulphocyanate mainly by preventing the marked and rapid increase in permeability which occurs at or shortly after the time of transfer to the pure salt solution.³ In the preceding paper I have adduced evidence that in anæsthesia the plasma membranes of irritable tissues are so modified as to render more difficult a *rapid* increase in permeability; hence stimulation, which involves this kind of change, is prevented. In the case of these egg cells the conditions appear to be essentially similar; the initial rapid increase in permeability, with the associated toxic action, is checked; but the more gradual alterations in the egg following prolonged exposure to the solution take place as before. Hence, a distinct antitoxic influence is evident as a rule only during the first hour or two in the solution; later this influence ceases to be perceptible. For the same reason the toxicity of sodium chloride solutions, which act gradually on Arbacia eggs, is not perceptibly counteracted by anæsthetics. These substances thus exhibit a characteristic contrast to such a salt as calcium chloride, as illustrated by the following instance. One hour's exposure to $m/2$ NaI typically kills all eggs; in the presence of the

³ Evidence of this effect is seen in the characteristic membrane formation and initiation of cleavage produced by brief exposure to these solutions. Cf. this Journal, 1910, xxvi, p. 106; 1911, xxvii, p. 289.

most favorable concentration of chloral hydrate (0.1 to 0.05 per cent in $m/2$ NaI) a minority of eggs — in my best experiments 20–30 per cent — may survive an exposure of two hours. Eggs left for this period in $m/2$ NaI containing a favorable proportion of calcium chloride are only slightly injured, and all form swimming larvæ when transferred to sea water and fertilized; a fair proportion of larvæ develop from eggs fertilized even after eighteen hours in such a solution. The salt thus exerts a far more lasting antitoxic influence than the anæsthetic. The difference, however, is one of degree; in both cases the basis of the antitoxic action is a modification of the plasma membrane, as my experiments with *Arenicola* larvæ clearly indicate; but apparently a modification due to changes in the condition of the lipoids alone is much less favorable than one in which all the colloids, including the proteins, are affected.

The fact that anæsthetics prevent a rapid increase of permeability and retard toxic action is a further indication that lipoids participate in the formation of the plasma membrane, and proves that the latter cannot be regarded simply as a modified protein film. The surface film of cells must contain — in accordance with Gibbs' principle — a certain proportion of every diffusible protoplasmic constituent which has any effect in lowering the surface tension of the cell boundary; lipoids, as well as proteins and other substances (varying with the specific composition of the protoplasm), must thus enter into its composition. The proportion of lipoids to proteins no doubt varies in the plasma membranes of cells, just as it is known to vary in different tissues. Hence anæsthetics affect different cells and tissues differently. In my own experiments I have found that the antitoxic action of anæsthetics is more pronounced, and can be demonstrated after a longer stay in the solutions, in the musculature of *Arenicola* larvæ than in sea-urchin or starfish eggs. Thus ether and other anæsthetics are unable to prolong the life of unfertilized *Arbacia* eggs in sulphocyanate or iodide solutions for more than one or two hours at most; while in *Arenicola* the recovery of muscular contractility in sea water, after many hours in 0.55 m NaCl containing ether, chloroform, or chlore-tone, is much more complete and rapid than after a similar stay in the pure solution. Another difference between the eggs and the larvæ is that definite antitoxic effects with chloroform or alcohol cannot (in my experience) be obtained with *Arbacia* eggs; while with *Arenicola* these

effects are well marked. These differences may be due to the existence of a higher lipid content in the plasma membranes of the contractile tissue than of the eggs. There are probably also qualitative differences of lipid content; at present what I wish to emphasize is that the existence of lipoids as essential constituents of the membranes is definitely indicated by the present experiments.

EXPERIMENTAL.

The experiments described below were performed at Woods Hole during the summer of 1911. The unfertilized eggs of both starfish (*Asterias forbesii*) and sea-urchins (*Arbacia punctulata*) were used. The experiments with each species of egg will be described separately.

Experiments with starfish eggs.—The unfertilized mature eggs of *Asterias forbesii* were used in these experiments, and the salt solution was in all cases isotonic sodium chloride (0.55 *m* NaCl). The procedure was as follows: The eggs, after removal from the animal, were left in sea water from two to three hours to allow time for maturation. Several cubic centimetres of a thick suspension of eggs were then brought into Erlenmeyer flasks of 50 c.c. capacity, the sea water was removed as far as possible with a pipette, and 50 c.c. of the solution added; after the eggs had settled to the bottom of the flask, as much as possible of the solution was withdrawn and replaced by 50 c.c. of fresh; the original small quantity of sea water introduced with the eggs is thus diluted at least a thousand times, so that its influence may be regarded as negligible. Where volatile anaesthetics were used the flasks were kept tightly corked. At intervals eggs were removed and examined in watch glasses. After remaining in the solutions for a period known to be sufficient for the pure salt solution to destroy all or nearly all of the eggs (one to two hours or more), the latter were transferred from each solution to a large quantity of sea water and washed free from salt and anaesthetic by one or more changes of sea water; spermatozoa were then added. The proportion of blastulae developing from eggs exposed to any solution, as compared with the proportion developing from the control eggs left in sea water, was taken as index of the injurious action of the solution.

Pure isotonic solutions of sodium chloride have a rapid destructive action on mature starfish eggs, causing agglutination and rapid increase

of permeability.⁴ In the course of one to two hours at normal summer temperature (20° – 24°) the protoplasm of such eggs assumes an opaque and coagulated appearance; swelling and disintegration follow rapidly. The same changes occur in immature eggs, though less rapidly, indicating the presence of a more resistant plasma membrane previous to maturation. These changes are to be regarded as primarily dependent on an increase in the permeability of the plasma membrane. A similar cytolysis preceded by protoplasmic coagulation occurs normally in unfertilized eggs left in sea water for a period of fourteen to eighteen hours. Oxidations play an essential part in this change, since it is greatly retarded in the absence of oxygen or in the presence of cyanide.⁵ The period during which the egg remains living and capable of developing after fertilization may thus be greatly prolonged by keeping in oxygen-free or cyanide-containing sea water. The cytolytic action of pure isotonic sodium chloride solution is also retarded by cyanide; the anticytolytic effect, however, is only moderate: in .55 *m* NaCl containing *m*/1000 KCN the great majority of eggs undergo coagulation within two to three hours. Calcium chloride has decidedly greater anticytolytic effect than cyanide. Anæsthetics in certain concentrations have a similar effect. The experiments summarized in Table I will illustrate:

Several other series of experiments gave similar results. Ether, chloroform, and chloral hydrate all showed in certain concentrations a definite and well-marked effect in retarding the cytolytic action of 0.55 *m* NaCl. Ether has a distinctly more favorable action than chloroform or chloral hydrate in the above concentrations. The two latter, while checking cytolysis, do not conserve the developmental power of the egg as ether does. Seven experiments in all were performed with chloroform in one sixth and one tenth saturated concentrations; the effects were similar to those described in the table: cytolysis was retarded at first, but the eggs on return to sea water after three hours in the solutions all failed on fertilization to form blastulæ. In two experiments with five volumes per cent ethyl alcohol a similar result was seen. The antitoxic action of ether and chloral hydrate was found to be exerted most favorably within a somewhat narrow range of concentrations, ranging in the case of ether from 0.7 vol. per cent to 0.4

⁴ Cf. this Journal, 1911, xxvii, p. 291.

⁵ J. LOEB: Archiv für die gesammte Physiologie, 1902, xciii, p. 59.

vol. per cent with an optimum at 0.5 or 0.6 vol. per cent, and in the case of chloral hydrate from 0.2 to 0.1 per cent, or possibly lower. Detailed descriptions of these experiments are omitted for lack of

TABLE I.

June 17. — Eggs of *Asterias forbesii*; the majority undergo apparently normal maturation. The fertilized eggs give numerous active gastrulæ. Unfertilized eggs were brought into the following solutions two hours after removal from the animals. After two and a half hours in the solutions they were transferred to sea water; after washing in sea water sperm was added.

Solution.	Condition of eggs in solutions after <i>ca.</i> two hours.	Effect of fertilization in sea water after two and one-half hours in solutions.
1. 0.55 <i>m</i> NaCl.	{ <i>Ca.</i> 95 per cent dead and coagulated; a few remain clear.	No development.
2. 100 v. 0.55 <i>m</i> NaCl + 5 v. <i>m</i> /2 CaCl ₂ .	All eggs clear and normal-looking.	Majority form blastulæ, largely abnormal.
3. 0.55 <i>m</i> NaCl + 0.3 per cent chloral hydrate.	Great majority dead and coagulated as in Solution 1.	No blastulæ.
4. 0.55 <i>m</i> NaCl + 0.6 v. per cent ether.	{ Majority coagulated, but a good many remain clear.	<i>Ca.</i> 10 per cent of mature eggs form blastulæ, largely abnormal.
5. 0.55 <i>m</i> NaCl + 0.4 v. per cent ether.	{ Most coagulated, but clear eggs more numerous than in Solution 4.	<i>Ca.</i> 20-30 per cent of mature eggs form blastulæ.
6. 0.55 <i>m</i> NaCl + 1/6 saturated chloroform	{ <i>Ca.</i> 50 per cent are translucent without membranes; remainder with membranes and coagulated.	No blastulæ.
7. 0.55 <i>m</i> NaCl + 1/10 saturated chloroform.	Great majority coagulated; a few remain clear.	Almost all die early; one blastula seen.
8. 0.55 <i>m</i> NaCl + <i>m</i> /1000 KCN.	<i>Ca.</i> 60 per cent remain clear; rest coagulated.	{ <i>Ca.</i> 5 per cent of mature eggs form abnormal blastulæ.

space. In one series in which eggs were fertilized after two hours in the solutions, most of the mature eggs which had been exposed to 0.55 *m* NaCl containing 0.75, 0.6, and 0.5 vol. per cent ether formed blastulæ; 0.4 and 0.3 vol. per cent solutions were less favorable,

though still distinctly effective; 0.2 vol. per cent had only slight effect. In the pure 0.55 *m* NaCl almost all of the eggs were dead and coagulated after two hours. Chloral hydrate showed well-marked protective action in 0.2 per cent, but not in stronger solutions. These results are typical. In five series of experiments with ether, in concentrations ranging from 0.75 vol. per cent to 0.2 vol. per cent, the most favorable antitoxic effects were seen in concentrations between 0.6 and 0.45 vol. per cent. With chloral hydrate the best concentrations were 0.2 or 0.1 per cent (four series ranging from 0.6 to 0.1 per cent). In sea-urchin eggs also chloral hydrate was found to act most effectively in low concentrations (0.1 to 0.05 per cent; see below).

Toward the end of June the starfish eggs began to exhibit abnormal behavior: the eggs even after maturation often showed an abnormally high resistance toward 0.55 *m* NaCl; such eggs also failed to undergo the normal coagulative change in sea water and were difficult to fertilize; the action of anaesthetics was also abnormal. The behavior of these eggs will not be considered in the present paper.

Experiments with *Arbacia* eggs.—In the experiments with unfertilized eggs of *Arbacia* the chief salts used were sodium and potassium iodide, sulphocyanate and nitrate. A few series of experiments were also performed with neutral and alkaline solutions of sodium chloride. In pure neutral 0.55 *m* NaCl, with an exposure of about three hours, there was no evident antitoxic action with ether (0.6 per cent), chloroform (one sixth saturated), ethyl urethane (0.3 per cent), chloretone (one tenth and one twentieth saturated), and chloral hydrate (0.1 and 0.05 per cent). On the other hand, calcium chloride (*m*/40) and potassium cyanide (*m*/1000) showed definite and well-marked action, particularly the former. Three hours' exposure to pure 0.55 *m* NaCl typically renders most eggs incapable of development, but a considerable proportion may survive; the toxic action of this salt is thus comparatively gradual, — hence, in all probability the absence of protective action with anaesthetics as explained above. The addition of alkali (*m*/500 NaOH) to the 0.55 *m* NaCl increases the toxicity of the solution, apparently by accelerating its permeability-increasing action; and in such alkaline solutions chloral hydrate and chloretone, and to a less degree ether, were found to exhibit well-marked antitoxic action. It is remarkable that calcium, so efficacious with the neutral solution, *increases* the toxicity of the alkaline solution. Otherwise

the results of these experiments correspond closely with those obtained with the more rapidly acting salts, as described below. The experiments with solutions of the other salts named above will now be described in order.

TABLE II.

July 20, 1911. — Unfertilized eggs of *Arbacia punctulata* were exposed to the following solutions for one and three-quarters hours; they were then transferred to sea water and sperm was added.

Solution.	Result (condition after sixteen hours).
1. $m/2$ NaI.	No development; eggs dead and partly decolorized.
2. 9 vols. $m/2$ NaI + 1 vol. $m/2$ CaCl_2	Most eggs form blastulæ, largely irregular; many surface swimmers.
3. $m/2$ NaI + $m/1000$ KCN.	Almost all are dead; largely disintegrated; only one blastula seen.
4. $m/2$ NaI + 0.6 v. per cent ether.	All dead and laked.
5. $m/2$ NaI + 0.2 per cent urethane.	A few blastulæ — less than 1 per cent.
6. $m/2$ NaI + 0.1 per cent urethane.	A few blastulæ — less than 1 per cent.
7. $m/2$ NaI + 0.05 per cent urethane.	A few blastulæ; somewhat more than in Experiments 5 and 6.
8. $m/2$ NaI + $1/8$ saturated chloretone.	A few blastulæ.
9. $m/2$ NaI + $1/16$ saturated chloretone.	A few blastulæ — less than 1 per cent.
10. $m/2$ NaI + 0.1 per cent chloral hydrate.	A fair number of blastulæ — more than in Experiments 5 to 9 — <i>ca.</i> 3-4 per cent.

Sodium iodide. — In solutions of this salt antitoxic effects were obtained with urethane, chloretone, and chloral hydrate, but not with ether or chloroform. The action of $m/2$ NaI is not readily counteracted, and the antitoxic effects with anæsthetics are relatively slight; in four out of eight series of experiments the anæsthetics showed no definite protective action. On the other hand, calcium and to a less degree cyanide are always highly effective. Table II summarizes the results of a favorable series of experiments.

In a second series on July 21 with ether (0.8, 0.6, and 0.5 vol. per cent) and chloral hydrate (0.1 and 0.05 per cent) no definite antitoxic

action was seen with ether, but with chloral hydrate this action was well marked; after two hours in the solutions of this anæsthetic, followed by return to sea water and fertilization, a considerable proportion of eggs — from 30 per cent to 40 per cent — formed blastulæ. Pure $m/2$ NaI — Kahlbaum's salt, dissolved shortly before using so as to be free from iodine — as a rule kills all of the eggs within an hour, though occasionally a few eggs may survive one and a half or even two hours' exposure. The above is the most decided antitoxic action shown by an anæsthetic in any of my experiments with this salt. Calcium is far more effective; in the above series almost all eggs formed blastulæ after two hours in a mixture of 9 vols. $m/2$ NaI plus 1 vol. $m/2$ CaCl_2 ; in another series a few eggs formed blastulæ after eighteen hours in the same mixture. Cyanide is decidedly less effective than calcium, though its action is perfectly distinct; in no case, however, did it prove so effectual as chloral hydrate in the experiments just cited.

Sodium sulphocyanate. — Two series of experiments were performed with this salt. In the first series all of the eggs failed to develop after an exposure of one hour and five minutes to pure $m/2$ NaCNS;⁶ while nearly all of those in the calcium-containing (plus $m/20$ CaCl_2) and about half of those in the cyanide-containing (plus $m/1000$ KCN) solutions formed blastulæ. On the other hand, ether (0.8 and 0.6 vol. per cent), urethane (0.4 and 0.2 per cent), chloretone (one tenth saturated), and chloral hydrate (0.1 and 0.05 per cent), while retarding cytolysis, did not enable the eggs to withstand this exposure. In the second series, with exposures of thirty minutes, urethane, chloretone, and especially chloral hydrate showed distinct protective action; with ether the effect was doubtful. A few eggs survived this exposure to the pure solution; the proportion of survivors was decidedly increased in the presence of the above anæsthetics. The extraction of pigment was distinctly retarded in those solutions where antitoxic action was evident, especially in those containing calcium and cyanide — indicating again that the antitoxic effect runs parallel with the prevention of abnormal increase of permeability.

Sodium nitrate. — Four series of experiments were performed with $m/2$ NaNO_3 with respective exposures of twenty minutes, one and a

⁶ The salt was of Kahlbaum's make and contained some alkali. The solutions were rendered neutral to phenolphthalein before using.

quarter hours, two hours five minutes, and three hours ten minutes. In the series with three hours' exposure no antitoxic action was apparent except with calcium and cyanide. With two hours' exposure chloral hydrate (0.1 and 0.05 per cent), chloretone (one tenth and one twentieth saturated), and urethane (0.4 and 0.2 per cent) showed distinct action; all eggs were destroyed in the pure $m/2$ NaNO_3 , while a considerable number survived and yielded larvæ in the presence of the anæsthetic, especially with 0.2 per cent urethane (40 to 50 per cent blastulæ) and one tenth saturated chloretone (30 to 40 per cent blastulæ). Ether (0.6 vol. per cent) showed no antitoxic action. Calcium and cyanide were markedly effective, as usual with sodium salts. The protective action was plainly correlated with a prevention of the increase of permeability as indicated by exit of pigment. Results essentially similar to the above were obtained in the other two series. In one experiment a few eggs survived an exposure of nineteen hours to $m/2$ NaNO_3 containing 0.1 per cent urethane. Only cyanide and calcium showed a comparable action. Ether showed a slight protective action in one experiment, but chloroform none.

Potassium salts.—In general, potassium salts in pure isotonic solution are decidedly less toxic to unfertilized *Arbacia* eggs than the corresponding sodium salts. Anæsthetics show definite antitoxic action with the iodide and sulphocyanate. Potassium nitrate is comparatively non-toxic; in two experiments a considerable number of eggs which had been exposed for nineteen hours to $m/2$ KNO_3 formed larvæ on fertilization; in correspondence with this slowness of action the antitoxic effects with anæsthetics are less distinct with nitrate than with the other two salts.

Potassium iodide.—Three separate series of experiments were performed with $m/2$ KI with exposures of one hour forty minutes, two hours fifteen minutes, and three hours, respectively. Table III gives the results of the second of these series.

The series with exposures of one hour and forty minutes gave an almost identical result except that the results with urethane were more favorable. The same solutions were used, except that 0.8 per cent ether was substituted for one twentieth saturated chloretone. The series with three hours' exposure also showed distinct antitoxic action with chloral hydrate and chloretone, but doubtful action with

urethane; the eggs exposed to the pure solution for this period gave less than 1 per cent of blastulæ; the proportion of blastulæ from eggs exposed to the anæsthetic-containing solutions was also small but dis-

TABLE III.

July 31, 1911. — Unfertilized eggs of *Arbacia* were exposed to the following solutions for two hours and fifteen minutes, then returned to sea water and fertilized.¹

Solution.	Result (condition after nineteen hours).
1. $m/2$ KI.	About 50 per cent form blastulæ.
2. 9 vols. $m/2$ KI + 1 vol. $m/2$ CaCl_2 .	Proportion of blastulæ somewhat greater than in Experiment 1.
3. $m/2$ KI + $m/1000$ KCN.	All dead and coagulated.
4. $m/2$ KI + 0.6 vol. per cent ether.	All dead and coagulated.
5. $m/2$ KI + 0.4 per cent urethane.	Ca. 40-50 per cent form blastulæ; rather less favorable than Experiment 1.
6. $m/2$ KI + 0.2 per cent urethane.	Ca. 50 per cent form blastulæ; surface swimmers more numerous than in Experiment 1.
7. $m/2$ KI + 1/10 saturated chlore-tone.	Marked improvement; 90 per cent or more eggs form blastulæ; better than Experiment 2.
8. $m/2$ KI + 1/20 saturated chlore-tone.	Marked improvement; like Experiment 7.
9. $m/2$ KI + 0.1 per cent chloral hydrate.	Improvement over Experiment 1, though less favorable than Experiments 7 and 8.
10. $m/2$ KI + 0.05 per cent chloral hydrate.	Ca. 90 per cent form blastulæ; essentially like Experiments 7 and 8.

¹ After ca. twenty hours in the solutions the eggs were found completely laked in Solutions 1, 3, and 4; partly laked in Solutions 5 and 6; while pigment was retained in Solutions 2, 7, 8, 9, and 10. The antitoxic action thus shows a general parallelism with the action in preventing increase of permeability of the egg to its own pigment.

tinctly higher — from 3 to 5 per cent. It is noteworthy that in all three series cyanide showed no protective action, but, on the contrary, increased the toxicity of the solution. The same was true of ether. Calcium chloride in all cases proved much less effective with this salt than with sodium iodide.

Potassium sulphocyanate. — With this salt seven series of experiments were performed, the results of which showed close agreement.

The periods of exposure ranged from forty-five minutes to three hours. Exposures of one hour twenty-five minutes and one hour forty minutes to pure $m/2$ KCNS killed all the eggs; a few survived exposures of one hour (*ca.* 30 per cent formed blastulæ), one hour five minutes (*ca.* 5 per cent blastulæ), one hour ten minutes (*ca.* 10 to 15 per cent blastulæ), and one hour fifteen minutes, (*ca.* 2-3 per cent blastulæ); and about half the eggs developed to blastulæ after an exposure of forty-five minutes to the pure solution.

Table IV summarizes the results of a series of experiments in which eggs were transferred to sea water and fertilized after one, two, and three hours' exposure to the solutions.

The results of the other series of experiments agreed essentially with the above. Chloroform showed no protective action in any experiment; alcohol also proved ineffective. Ether and especially chloral hydrate showed marked antitoxic effectiveness in certain concentrations; the optimum for chloral hydrate was 0.1 to 0.05 per cent; the above result was the best obtained with ether. Ethyl urethane, in a series of experiments with this anæsthetic in the concentrations 0.4, 0.3, 0.2, and 0.1 per cent, also exhibited marked antitoxic action in solution of this salt. After one hour in pure $m/2$ KCNS, from 10 to 15 per cent of eggs formed blastulæ on fertilization, while of the eggs exposed to the same solution containing 0.4, 0.3, and 0.2 per cent urethane, from 75 to 90 per cent formed active blastulæ; in the 0.1 per cent solution there was no distinct antitoxic action. Chloretone was also used in this series, in concentrations of one tenth, one fifteenth, and one twentieth saturated, but showed no definite effects.

In a second similar series, with ether (0.6 vol. per cent), urethane (0.3 and 0.2 per cent), and chloretone (one tenth, one twentieth, and one thirtieth saturated), and exposures of one hour forty minutes, none of the eggs reached a larval stage; this exposure was evidently too long.

It is again to be noted that with this salt, as with potassium iodide, calcium proved decidedly less effective as antitoxic agent than with the corresponding sodium salt; also that potassium cyanide increased rather than decreased the toxicity of the solution.

Potassium nitrate. — Solutions of this salt are decidedly less toxic than those of iodide and sulphocyanate, and in the four series of ex-

TABLE IV.

July 7, 1911. — Unfertilized eggs of *Arbacia* were placed in the following solutions. Portions were transferred to sea water after respectively one, two, and three hours in the solutions; to each lot sperm was then added. The condition of the eggs next day is indicated in the table.

Solution.	Eggs exposed to solutions one hour.	Eggs exposed to solutions two hours.
1. .55 <i>m</i> KCNS.	{ About one third form mostly irregular blastulæ.	All dead and coagulated.
2. 9 vols. .55 <i>m</i> KCNS + 1 vol. <i>m</i> /2 CaCl.	Great majority form blastulæ.	{ About 75 per cent form blastulæ [of eggs exposed three hours very few form blastulæ].
3. .55 <i>m</i> KCNS + <i>m</i> /1000 KCN.	Few blastulæ — <i>ca.</i> 2-4 per cent.	All dead.
4. 55 <i>m</i> KCNS + 0.8 vol. per cent ether.	Great majority form blastulæ.	Nearly all dead; <i>ca.</i> 1 per cent blastulæ.
5. .55 <i>m</i> KCNS + 1 v. per cent ether.	A few blastulæ — <i>ca.</i> 3-4 per cent.	All dead.
6. .55 <i>m</i> KCNS + 1/6 saturated chloroform.	All dead and coagulated.	All dead.
7. .55 <i>m</i> KCNS + 1/8 saturated chloroform.	All dead.	All dead.
8. .55 <i>m</i> KCNS + 5 vol. per cent ethyl alcohol.	A few blastulæ — less than 1 per cent.	All dead.
9. .55 <i>m</i> KCNS + 0.2 per cent chloral hydrate.	A few blastulæ — <i>ca.</i> 2-3 per cent.	A few blastulæ — <i>ca.</i> 2 per cent [eggs exposed three hours all dead].
10. .55 <i>m</i> KCNS + 0.1 per cent chloral hydrate.	From 35 to 50 per cent form mostly irregular blastulæ.	{ <i>Ca.</i> 20-25 per cent form blastulæ [eggs exposed three hours give a few blastulæ — less than 1 per cent.]

periments tried the anæsthetics showed little antitoxic action. The following series (Table V), with an exposure of two hours, shows definite action with chloral hydrate, urethane, and chloretone. A few eggs exposed for twenty hours to solutions containing 0.2 per cent

urethane and one twentieth saturated chloretone survived and yielded larvæ on fertilization, while none of those similarly exposed to the pure solution developed.

TABLE V.

July 18, 1911. — Unfertilized eggs of *Arbacia* were placed in the following solutions. Eggs were transferred to sea water after periods of two and twenty hours and fertilized. The results were as follows.

Solution.	Result (condition the day after fertilization). Two hours' exposure.	Result (condition the day after fertilization). Twenty hours' exposure.
1. $m/2$ KNO_3 .	From half to two thirds form blastulæ.	All dead and coagulated.
2. 9 vols. $m/2$ KNO_3 + 1 vol. $m/2$ CaCl_2 .	Essentially like Experiment 1.	All dead.
3. $m/2$ KNO_3 + $m/1000$ KCN .	5-10 per cent form blastulæ.	All dead.
4. $m/2$ KNO_3 + 0.6 vol. per cent ether.	{ Majority dead; <i>ca.</i> 20-25 per cent form blastulæ.	All dead.
5. $m/2$ KNO_3 + 0.1 per cent chloral hydrate.	{ More favorable than Experiment 1; <i>ca.</i> 70 per cent to 85 per cent form blastulæ.	All dead.
6. $m/2$ KNO_3 + 0.05 per cent chloral hydrate.	{ Like Experiment 5.	All dead.
7. $m/2$ KNO_3 + 0.4 per cent urethane.	{ Great majority form blastulæ — <i>ca.</i> 85 per cent.	All dead.
8. $m/2$ KNO_3 + 0.2 per cent urethane.	<i>Ca.</i> 70 per cent to 80 per cent form blastulæ.	{ A small proportion form blastulæ — <i>ca.</i> 1 to 2 per cent.
9. $m/2$ KNO_3 + 1/10 saturated chloretone.	Almost all (more than 90 per cent) form blastulæ.	All dead.
10. $m/2$ KNO_3 + 1/20 saturated chloretone.	Almost all form blastulæ.	{ A small proportion form blastulæ — <i>ca.</i> 1 per cent.

The experiments with potassium nitrate again illustrate the comparative ineffectiveness of calcium as antitoxic agent with potassium as compared with sodium salts; also the unfavorable action of potas-

sium cyanide in solutions of potassium salts. This contrast between sodium and potassium salts has appeared through all of my experiments with unfertilized *Arbacia* eggs. Pure solutions of potassium salts are characteristically less toxic than those of the corresponding sodium salts; but in the presence of a moderate proportion of calcium chloride the relations are reversed. Sodium salts appear to be distinctively characterized by the readiness with which the toxicity of the pure solutions may be decreased by the addition of calcium; while in solutions of potassium salts the antitoxic action of calcium is relatively slight. Somewhat similar conditions are exhibited by *Arenicola* larvæ, which also withstand prolonged exposure to $m/2$ KCl better than to $m/2$ NaCl; calcium chloride has a markedly antagonistic action to sodium chloride, but relatively slight to potassium chloride.⁷ Cyanide also shows a remarkable difference in its action on unfertilized *Arbacia* eggs in solutions of sodium as compared with potassium salts. The immediate toxic action of sodium salts is decreased in the presence of $m/1000$ KCN, while that of potassium salts is distinctly and often markedly increased. I am unable at present to assign any grounds for this difference; possibly it indicates that sodium and potassium salts act primarily on different constituents of, or on different processes in, the plasma membrane. Loeb has found that the toxic action of many substances and agencies⁸ upon fertilized sea-urchin eggs is retarded by cyanide, and he has referred the antitoxic action in such cases to a suppression of abnormal or destructive oxidation processes. If the toxic action of salt solutions is due to the abnormal changes which they produce in the plasma membrane, the above facts seem to indicate that oxidations are in some manner associated with changes in the permeability of the membrane. Suppression of oxidative processes prevents pure solutions of sodium salts from increasing the permeability of the plasma membrane as they would otherwise do, and hence diminishes the toxicity of these solutions. But why cyanide fails to exercise this influence in solutions of potassium salts remains unexplained on any such hypothesis. It must be recognized that toxic effects may be the expression of a variety of alterations in the

⁷ Cf. this Journal, 1911, xxviii, p. 213.

⁸ Neutral salt solutions, hyperalkaline solutions, sugar solutions, hypertonic and hypotonic solutions, narcotics. Cf. J. LOEB: *Biochemische Zeitschrift*, 1910, xxix, p. 80.

living system. Hence an antitoxic agency which is effective with one form of toxic action may be quite ineffective with another. Further consideration of this question is deferred for the present.

SUMMARY.

The main facts and general conclusions of the foregoing paper may be briefly summarized as follows:

1. The permeability-increasing and cytolytic action of isotonic solutions of neutral sodium and potassium salts on unfertilized starfish and sea-urchin eggs is retarded in the presence of various anæsthetics in certain definite concentrations. The period during which the eggs remain living in the salt solutions, and capable of development after fertilization, may thus be greatly prolonged. This protective or antitoxic influence is comparable to that exercised by calcium or magnesium salts, though as a rule it is less marked.

2. The view that lipoids enter into the formation of the plasma membrane thus receives confirmation. Evidently the state of the lipoids determines the readiness with which the membrane undergoes alteration. The conditions suggest that the lipoids have a "protective" relation to the other colloids of the membrane, limiting or preventing changes of aggregation state in these latter, and that during anæsthesia this influence is increased.

3. Calcium chloride is much less effective as antitoxic agent with potassium than with sodium salts.

4. Potassium cyanide in $m/1000$ concentration, while decreasing the immediate toxic action of sodium salts on sea-urchin eggs, increases that of potassium salts.

THE INFLUENCE OF MUSCULAR ACTIVITY UPON THE ALVEOLAR TENSIONS OF OXYGEN AND OF CARBON DIOXID.

By THEODORE HOUGH.

[From the Physiological Laboratory of the University of Virginia.]

HISTORICAL.

IN connection with other investigations now in progress in this laboratory, it became desirable to have more complete information than is at present available on the influence of different degrees of muscular activity on the alveolar tensions of oxygen and carbon dioxid. The results are herewith briefly communicated because of their value in confirming previous work on one part of the subject, the tension of carbon dioxid; and also because they extend our knowledge by the information given on the oxygen tensions.

Previous investigation of the subject is limited chiefly to that of Haldane and his co-workers.¹ Haldane and Priestley determined the carbon dioxid tensions during work on a stationary bicycle. The amount of work done was very moderate, varying from 1880 to 4850 foot-pounds per minute, the respiratory exchange being quadrupled. The alveolar percentages of carbon dioxid increased from 5.62 to 5.75 in one subject and from 6.28 to 6.72 in the other. It may be said here that the maximum work done in these experiments was probably only slightly in excess of that represented by the *walk* given in this paper, and it will be seen that my results agree with Haldane's for this amount of work.

Miss Fitzgerald measured the alveolar carbon dioxid percentages just before and just after a bicycle ride of nine miles in fifty-three minutes and found the mean percentage before the ride to be 4.74, and after 4.72. She made only one experiment.

¹ HALDANE and PRIESTLEY: *Journal of physiology*, 1905, xxxii, p. 225; FITZGERALD and HALDANE: *Ibid.*, 1905, xxxii, p. 486; DOUGLAS and HALDANE: *Ibid.*, 1909, xxxviii, p. 420.

Pembrey and Cook² studied the alveolar percentages of oxygen and carbon dioxid, especially in relation to the phenomena of second wind. The brief report of their work in the Proceedings of the Physiological Society gives only incomplete details of experimental method, nor does it state whether the subject went immediately from the condition of rest to that of vigorous exercise or whether there was any period of "warming up." I think this is a point of considerable importance in estimating the significance of their results. I shall return to this point later in this paper; here we need only say that Pembrey and Cook found a rise of the alveolar carbon dioxid percentage during the initial respiratory distress by amounts varying from 0.44 to about 2.0 per cent. Later, with the advent of second wind, there was in most cases a tendency to return toward normal. The oxygen tensions uniformly showed a fall.

By far the most careful and extensive investigation of the subject is that of Douglas and Haldane (*loc. cit.*). In the case of very sudden and violent activity (running rapidly up, down, and up again a flight of stairs 40 feet high, the work being done at the rate of 30,000 foot-pounds or 4000 kgm. per minute) it was found that "the immediate effect of the exertion was to raise the alveolar CO₂ pressure by about 10 mm. in the first experiment, but that in successive experiments this rise was less, and that the rise was followed by a fall which reached its maximum about ten minutes after the exertion and was greater after each successive exertion." It will be seen that my results agree with this statement, with the exception of the initial rise, of which more later.

A long cross-country run of about eight miles in fifty-seven minutes gave the same general picture, although it was found that after this prolonged (but less intense) exertion the changes observed were not greater than after running twice up the laboratory stairs.

The previous inhalation of oxygen was without effect on the secondary fall of carbon dioxid pressure, as was also previous forced breathing, although in the latter case the respiratory distress was lessened. Finally, the after effects of severe exercise (secondary fall of carbon dioxid tension) were not observed after a walk of 600 yards at four miles per hour.

Similar secondary falls of carbon dioxid tension and *rise* of oxygen

PEMBREY and COOK: *Journal of physiology*, 1908, xxxvii, p. lxvii.

tension were found by Hill and Flack,³ who also show that, although the hyperpnea was sometimes accompanied by a marked rise of rectal temperature, it is also seen at times when the rectal temperature was little above normal. They conclude that the chief cause of the hyperpnea is in the stimulating action of the muscular metabolites, as suggested by Geppert and Zuntz. Their experiments were made on medical students during the London Hospital and Interhospital Athletic Sports and were made during the period of distress immediately following the races.

EXPERIMENTAL METHODS.

The method of obtaining samples of the alveolar air is essentially that of Haldane. Certain modifications in the method of collecting the sample require some attention.

The subject breathes through the mouthpiece of the respiration valves described and figured by the author in a previous paper.⁴ A pair of Müller's valves is provided with an additional pair of membrane valves to secure the separation of inspired and expired air. Just beyond the mouthpiece two short side tubes lead to the collecting burettes, which, with the connecting tubing, are filled as far as the mouthpiece tube with acidulated water (0.1 per cent HCl). Immediately beyond the sampling tubes the short piece of wide flexible rubber tubing leading to the valves is provided with a brass compress. It is only necessary to press upon this to separate the portion of the tube connected with the sampling burettes and mouthpiece from that connected with the valves beyond. In order to obtain the alveolar air at any desired phase of respiration, the subject breathes out rapidly, and, while this outward current of air is flowing, closes the compress after some 500 c.c. of air have been expelled. The dead space of the subject and of the tube as far as the compress are now filled with alveolar air at the time of the forced exhalation. A sample of this — usually about 60 c.c. — is then siphoned off into the collecting burette.

The gas thus collected is transferred, with the usual precautions, to a water-jacketed measuring burette, and first its carbon dioxide and then its oxygen determined by the use of absorption pipettes. The

³ HILL and FLACK: *Journal of physiology*, 1907, xxxvi, p. xi.

⁴ HOUGH: *this Journal*, 1910, xxvi, p. 156.

carbon dioxid was absorbed by 20 per cent sodium hydrate and the oxygen by phosphorus. After reduction to standard conditions, the alveolar tensions were calculated on the assumption that the alveolar air is saturated with aqueous vapor at 37° C. In practically all cases samples were taken of the alveolar air at the end of inspiration and at the end of expiration.

Four different degrees of muscular work were used: (1) that involved in the sitting posture which had been maintained for at least twenty minutes and had been preceded by no more vigorous work than is involved in walking about the laboratory; (2) that involved in walking for ten or more minutes at the rate of four miles an hour; (3) that involved in a trot which was rapid enough to produce *hyperpnea*, but not labored breathing; (4) that involved in a hard run (usually a "run in place") which produced decidedly labored breathing. The samples of air were taken within ten to twenty seconds after cessation of the exercise, or else at varying intervals of time (fifteen to forty minutes) afterwards.

It may be well to remind the reader that the so-called "inspiratory" and "expiratory" samples cannot be taken on the same respiratory movement; indeed, thirty seconds were allowed to elapse between the two to permit the effects of the modification of the respiratory movements necessary in taking one sample to pass off before the next sample was taken. In the case of the samples taken immediately after various forms of exercise the inspiratory sample was first taken, after which the exercise was continued for one minute or more before taking the expiratory sample. In other cases the order of taking the two samples was reversed. Considerations of these necessary conditions of the experiments will explain why the carbon dioxid tension is sometimes lower or the oxygen tension higher in the expiratory than in the inspiratory sample. Inspection of the tables will show that this does not happen frequently.

In the trot and the run care was taken to work up gradually to the desired intensity of the work. This period of "warming up" lasted from two to five minutes, according to the final intensity of exercise desired. Time was thus given for the circulation to adjust itself to the new conditions, after which the work was continued at approximately uniform intensity for the period given. This was usually from seven to ten minutes.

In concluding this description of the experimental procedure, the writer desires to emphasize the necessity of the utmost accuracy in collecting the samples of alveolar air, being convinced from his own experience that herein lies a great chance for error. The forced expiration which precedes siphoning off the sample must be made as rapidly as possible and *in no case can it be in the least prolonged*. It is obvious that even a relatively slight prolongation of this expiratory effort will give too high values for carbon dioxid and too low values for oxygen. This consideration applies in general to all collections of samples of alveolar air, but is doubly important under the conditions of muscular activity when the blood is bringing back greatly increased quantities of carbon dioxid and carrying away equally increased quantities of oxygen. A prolongation of two or three seconds of the expiratory effort before taking the sample may easily lead — as I know from actual determinations — to an increase of 5 or 10 mm. of carbon dioxid tension and an equal decrease of the oxygen tension. Even when on one's guard against the error, one does not always succeed in avoiding it, especially when in respiratory distress. *No subject of experiment is reliable until he has had very considerable practice in taking these samples and even in taking them under the conditions of hard muscular activity*, and if he understands what he is doing, so much the better. I believe that the introduction of the compress already described is, for this reason, a material improvement over Haldane's original method; and I am inclined to suspect that the differences between my results and those of Pembrey and Cook and some of those of Douglas and Haldane may be attributable to this difference in our experimental procedure. In other words, in Haldane's method one must not only force through the long tube (4 feet long, 1 inch in diameter) some 500 c.c. of air, but must also breathe out some 300 c.c. more of air to fill this tube. When the breathing is very deep and rapid, it is often difficult to secure this 700-800 c.c. of air at the end of expiration. With the writer's modification it is only necessary to force out the 500 c.c. before closing the compress, and there is no temptation to prolong the expiratory effort beyond this point.

RESULTS.

The results of the experiments are given for the greater part in Tables I and II. All these experiments were made on the same sub-

ject, although a few on other subjects show that the figures in the tables are qualitatively representative. The results are expressed in terms of alveolar tensions (mm. of mercury). The figures in Table I are from samples taken immediately (ten to twenty seconds) after the muscular activity; those in Table II from samples at varying intervals after the exercise, the subject sitting still, or at most only transferring gas from the collecting to the measuring burette between the exercise and the collection of the sample, and always sitting still for several minutes before taking the sample.

1. *Alveolar tensions immediately after muscular activity.* — The sitting (or resting) tensions show the constancy of that of carbon dioxid, first pointed out by Haldane. It is interesting to note that the two tensions of this gas which are distinctly lower than the rest were observed on exceptionally warm days, thus agreeing with the observations of Boycott and Haldane⁵ on the effect of external temperature on alveolar tension. The figures also agree with Haldane's observation of the greater variability of the oxygen tensions.

We may also note in our results the agreement with Haldane's observation of the increasing difference between inspiratory and expiratory tensions, as the intensity of the muscular work increases. Thus the differences for carbon dioxid are 2, 3, 4.3, and 7.5 mm. Hg, and for oxygen 0.2, 6.0, 7.0, and 9.3 mm. Hg, for our four grades of muscular work. The obvious explanation is that given by Haldane, namely, the greater depth of the breathing movements.

Passing now to the quantitative changes observed, we note, as the most striking feature in the alveolar tensions *immediately after the exercise*, that the walk produces a small rise of the carbon dioxid tension, the trot shows about the same tension as the walk or rather a tendency to a lower tension, while with the hard run there is a distinct fall from that of the trot or the walk, and that this fall usually goes below that of the resting tension.

For the four experiments in which we can directly compare the tensions in the sitting posture and after the walk, the rise of carbon dioxid tension is only between 1 and 2 mm. of mercury on an average, but it seems to be the rule. There is, of course, a considerable increase of production of carbon dioxid during the walk, and our figures show that the increased ventilation of the lungs almost, but not quite, keeps

⁵ BOYCOTT and HALDANE: *Journal of physiology*, 1908, xxxvii, p. 358.

TABLE I.

ALVEOLAR TENSIONS TEN TO TWENTY SECONDS AFTER MUSCULAR ACTIVITY; CO₂.

Date, 1911.		8,21	8,24	8,25	8,31	9,1
Sitting	Insp.	33.4	33.0	35.7	35.7	38.4
		104.9	98.2	88.3	105.5	102.9
	Exp.	37.5	33.7	39.4	38.4	39.2
		87.6	103.8	106.7	100.5	100.9
	Av.	35.5	33.4	37.6	37.1	38.8
		96.3	101.	97.5	103.	101.9
Walk	Insp.	38.8
		115.4
	Exp.	45.4
		101.7
	Av.	42.1
		108.6
Trot	Insp.	39.1	38.3	37.5	36.9
		103.8	105.3	99.3	112.3
	Exp.	39.6	40.5	46.4	42.2	43.9
		102.5	94.7	102.1	94.9	98.5
	Av.	39.4	39.4	39.8	40.4
		103.3	100.	97.1	105.4
Run	Insp.	29.6	34.7
		123.8	117.2
	Exp.	42.2	40.5
		108.7	104.3
	Av.	33.4	37.6
		116.3	110.8

TABLE I.

ROMAN; O₂, ANTIQUE. EXPERIMENTS MADE ON THE SAME DAY ARE SUCCESSIVE.

9,2	9,4	9,5	9,25	9,29	10,2	10,25	Av'ge.
36.8	38.2	37.0	36.0
96.5	103.4	103.5	100.4
39.1	38.7	38.2	38.0
96.4	107.1	101.8	100.6
37.9	38.4	37.6	37.0
96.5	105.2	102.7	100.5
37.1	40.6	35.3	38.0
104.3	100.9	107.1	106.9
41.1	39.3	38.2	41.0
93.9	102.9	105.2	100.9
39.1	40.0	36.6	39.5
99.1	101.9	106.7	103.9
35.6	35.0	39.9	34.9	38.9	39.2	37.5
102.9	123.7	106.8	110.3	106.3	106.0	107.7
42.	42.1	41.7	37.0	43.2	43.2	41.8
97.2	115.8	100.8	107.9	94.8	98.5	100.7
38.8	39.6	40.8	36.0	41.1	41.2	39.7
100.0	119.7	103.8	109.1	100.6	102.3	104.2
....	27.1	33.8	31.1	31.3
....	124.2	111.1	113.6	118.0
....	33.0	40.6	37.7	38.8
....	120.9	104.9	104.6	108.7
....	30.1	37.2	34.4	35.0
....	122.6	108.0	109.1	113.3

TABLE II.
ALVEOLAR TENSIONS AT DIFFERENT PERIODS OF TIME AFTER MUSCULAR ACTIVITY. CO₂, ROMAN; O₂, ANTICQUE. FIGURES IN PARENTHESES
GIVE THE TIME ELAPSING BETWEEN THE CESSATION OF WORK AND TAKING THE SAMPLE.

Date, 1911.		After run of seven to ten minutes.					After trot of ten minutes.		
		10,2	10,6	10,20	10,23	Avg'e.	10,25	11,10	Avg'e.
After two to five min.	Inspiration. . .	25.7 129.7 (2)	24.0 132.5 (2-3)	26.3 117.8 (2)	25.3 126.6	34.0 110.9 (5)	35.0 111.2 (5)	34.5 111.1
	Expiration. . .	28.2 122.6	26.1 127.2	25.4 127.2	26.5 125.3	35.8 107.6	38.3 99.6	37.1 103.6
	Average. . .	26.8 126.2	25.0 129.9	25.8 122.5	25.9 126.0	34.9 109.8	36.7 105.4	35.8 107.4
After seven to ten min.	Inspiration. . .	32.1 111.9 (7)	27.4 116.4 (10)	27.4 112.4 (8)	28.2 112.3 (11)	27.7 113.7
	Expiration. . .	32.2 109.2	32.9 103.2	27.1 111.0	28.0 117.6	29.3 110.6
	Average. . .	32.1 110.6	30.2 109.8	27.3 111.7	28.1 115.0	28.5 112.2
After fifteen to twenty min.	Inspiration.	27.8 96.8 (20)	24.6 113.9 (20)	26.9 121.2 (23)	26.4 110.6	31.9 115.7 (15)	34.7 98.6 (17)	33.3 107.1
	Expiration.	23.7 107.8	24.8 117.2	28.3 116.0	25.6 113.7	34.9 110.6	37.2 104.6	36.0 107.6
	Average.	25.8 102.3	24.7 115.6	27.6 118.6	26.0 112.0	33.4 113.2	35.9 101.6	34.7 107.4
After thirty to forty min.	Inspiration.	28.5 119.7 (40)	33.3 108.0 (30)
	Expiration.	32.1 116.6	35.1 107.1
	Average.	30.3 118.4	34.2 107.6

pace with this. The alveolar oxygen tension at the same time shows a rise from an average of 101.6 to 104 mm. (in the four experiments in question). The increased ventilation of the lungs which almost keeps pace with the increased production of carbon dioxide is more than enough to care for the increased consumption of oxygen.

The same four experiments give the alveolar tensions after a trot — which, it will be remembered, produced hyperpnea but not labored breathing — and these show little change from those taken after the walk. The average carbon dioxide tension after the walk is 39.5 mm.; that after the trot is 39.9. In the first three out of the four experiments, however, there is a trifling fall in the average tension. The increased ventilation of the lungs in these cases more than counterbalances the increased production of carbon dioxide. That the respiratory movements now slightly overventilate the lungs is furthermore suggested by the rise of the alveolar tension of oxygen from 104 (walk) to 107 (trot).

The trot thus shows the suggestion of an overventilation of the lungs. With the increased muscular activity represented by the hard run, this overventilation becomes much more pronounced. The figures in the column of averages in Table I represent with more than usual accuracy what takes place in each experiment. The average carbon dioxide tension falls from 39.7 to 35, while the oxygen rises from 104.2 to 113 mm.

The term "overventilation" is used in the above merely to indicate that, so far as the alveolar tensions are concerned, the respiratory movements more than keep the alveolar tensions of the two gases normal; they send the carbon dioxide tension below the normal, and to an even greater extent they send the oxygen tension above the normal. It may be noted that, so far as it goes, the effect must be to increase the difference of pressures in the blood and the alveoli for both gases at the very time that the increased speed of the blood through the lungs requires more rapid diffusion.

In the experiments of September 25 and 29 the first run of ten minutes was followed immediately after taking the samples given in Table I by a second run of ten minutes. The samples taken immediately after this second run show a still further fall of the carbon dioxide tension and an average rise of the oxygen tension (see Table III). It is evident that the longer this severe muscular activity is continued the more pronounced do the changes of alveolar tensions become.

2. The after-effects of muscular activity upon alveolar tensions. — Table II gives the tensions in samples taken at varying periods after the cessation of the muscular work. Part of the results of the experiments of October 2 and October 25 will be found in Table I.

TABLE III.

SHOWING THE INFLUENCE OF THE DURATION OF THE EXERCISE UPON THE ALVEOLAR TENSIONS: I, TENSIONS AFTER FIRST TEN MINUTES OF THE RUN; II, TENSIONS AFTER SECOND TEN MINUTES OF THE RUN.

Breathing.	Sept. 25.		Sept. 29.	
	I.	II.	I.	II.
Inspiration . .	{ 27.1 124.2	{ 20.3 130.0	{ 33.8 111.1	{ 30.4 117.5
Expiration . .	{ 33.0 120.9	{ 31.9 112.7	{ 40.6 104.9	{ 35.2 110.1
Average . . .	{ 30.1 122.6	{ 26.1 121.4	{ 37.2 108.0	{ 32.8 113.8

The results need no extended comment, as they speak clearly for themselves. During the second or third minute after the hard run of ten minutes there is a marked fall of the tension of carbon dioxid and an even more striking rise of the tension of oxygen. Thus the averages give a fall from 35 mm. to 26 mm. in the former case and an increase from 113 mm. to 126 mm. in the latter. The overventilation of the lungs apparent at the cessation of the work becomes during this period more pronounced. This result, we shall see in what follows, is capable of simple explanation.

As to the further course of this alveolar condition, our experiments show that the carbon dioxid tension was lowest about two to three minutes after work, then rose by some 3 mm. by the tenth minute, after which there was a second fall in all experiments, as shown by the analyses about twenty minutes later. Our experiments are too few in number to say whether this uniformity of result is accidental or not. If it is the rule, the writer has no explanation which he can suggest with any degree of confidence.

In two experiments the alveolar tensions were determined at periods of five, fifteen, and thirty minutes after the trot (Table II). As far as they go they show qualitatively the same course as those taken after the hard run, although they are quantitatively less in amount. Here, again, the effect lasts for at least thirty minutes; indeed, at this time our figures show the same deviation from the normal as is seen after the fifth minute. It is significant that both in the case of the trot and the run there seems to be little change in this condition of over-ventilation for half an hour after the exercise.

DISCUSSION OF RESULTS.

In speaking of the effects of muscular activity upon any function of the body one must distinguish carefully between those primary effects which are directly attributable to the events taking place in the contracting muscles and those secondary effects caused by various conditions introduced into the organism by the work. For example, the excessive rise of systolic blood pressure in the brachial artery which McCurdy demonstrated to be caused by the maximal lifting effort involved in the dynamometer test for strength of legs is really due, as McCurdy points out, to the pressure conditions suddenly introduced into the pleural space by a forcible contraction of thoracic and abdominal muscles against a closed glottis; they do not represent the immediate effect of the contracting leg muscles on arterial pressure.

The need of making this distinction is even more clear in the case which we are investigating. Certain forms of muscular work interfere with the free movement of the ribs and diaphragm and, by the resulting underaeration of the lungs, influence the gaseous tensions both of the alveoli and of the blood in special ways. Constricting clothing, the closure of the glottis in exercises of maximal effort, the fixation of the ribs to afford firm origin to working muscles, breathing through the nose instead of through the mouth when the volume of air respired is greatly increased, are obvious examples of such secondary influences of muscular activity upon respiratory conditions.

I would stress this point here because I am inclined to think that some forms of exercise used by previous observers may have introduced these very conditions of interference with the perfectly free movements of ribs and diaphragm. The rapid running up and down stairs in the

experiments of Douglas and Haldane seems to the writer open, to some extent, to this criticism; and the fatigue of the respiratory muscles in sprinting or even in cross-country running may also interfere with full pulmonary ventilation. In other words, while observations on the respiratory mechanism under the conditions in question are valuable as showing what effects may be produced there, directly or indirectly, by certain forms of muscular work, they have little or no value as showing the response of the respiratory centre under the conditions in question, for the work of this centre can be measured only by observations on the skeletal and muscular mechanisms of respiration *when these mechanisms are free to respond to the full extent to the stimulus received from the centre.*

In the experiments given in this paper the utmost care has been used to guard against this fundamental source of error. Constricting clothing has been avoided; during hyperpnea mouth breathing has been used whenever nasal breathing offered any obvious resistance to the entrance or exit of air, and, finally, the forms of exercise chosen in no way impede the free action of the thorax.

The writer also believes that the modification of Haldane's method of taking the sample of alveolar (introduction of the compress) materially contributes to the accuracy of results. The advantages of this improvement have already been fully dwelt upon, and I need only add that all samples were rejected when the experimenter (who was also the subject) was not sure that there had been no prolongation of the forced expiration. It must never be forgotten that any such prolongation of this expiration is sure to lead under the conditions of hard muscular work to erroneously high values for carbon dioxid and low values for oxygen.

The above sources of error are urged with reference especially to the one point of difference between our results and those of most other workers. All agree upon the lowering of the carbon dioxid tension when the hyperpnea has largely passed away five or more minutes after the exercise. Several observers, on the other hand, find that the sample taken within ten or twenty seconds after the cessation of vigorous work shows a considerable rise of carbon dioxid tension, especially if the subject is "winded." The writer has uniformly failed to get this rise. The samples taken immediately after ten minutes of hard work invariably showed a fall, which was greater the more intense the work

and also increased with the duration of the work. The experiments also show that the changes of carbon dioxid tension go along with corresponding changes of opposite character in the oxygen tensions, thus supporting the view previously expressed that they represent an overventilation of the lungs.

This overventilation of the lungs is fully explained on the assumptions that during work in which a muscle or group of muscles contracts with any degree of intensity we are dealing with the entrance of a second stimulus⁶ to the respiratory centre in addition to the ordinary stimulus of carbon dioxid; that this stimulus is some katabolite or katabolites of the working muscles; that it is slowly eliminated from the blood and hence continues to exert its stimulating influence for half an hour or more after the cessation of the muscular work.

Putting all the facts together, it would seem that they are satisfactorily explained as follows. During very moderate muscular activity, such as that of a walk, the increased breathing is the result of a slightly increased tension of carbon dioxid in the arterial blood. Our figures show in each experiment an increase of from 1 to 3 mm. This is caused by the greater production of carbon dioxid by the muscles which destroys the equilibrium which had previously existed between the production of this gas by the body and its elimination by the existing lung ventilation. The tension of the gas rises in the arterial blood, and this stimulates the centre to greater activity until a new equilibrium point is established. After the cessation of such work, with the diminished production of carbon dioxid by the muscles, the old equilibrium is rapidly re-established and the carbon dioxid tension does not go below normal (Douglas and Haldane).

With greater intensity of work the effect of the muscular katabolites adds itself to that of the carbon dioxid, and the overventilation of the lungs begins. This tends to decrease the carbon dioxid tension and to raise that of oxygen, thus opposing the tendency of the increased production of carbon dioxid to produce the opposite effect. The result is the algebraic sum of the two factors, and in the case of the trot we find that there has usually been little change of alveolar

⁶ The word "stimulus" is here used in the sense merely of increasing the activity of the centre and without prejudice to the possible differences in mode of action which Haldane assumes for the various substances which would meet the terms of this definition.

tension from that observed during the walk. With the still more vigorous work of the run, the effect of the overventilation gets the upper hand, and the alveolar carbon dioxid tension goes below, while that of oxygen rises above normal.

These changes in the alveoli are probably greater than those in the blood. It would seem that three factors determine the arterial carbon dioxid tension: the tension of this gas in the lungs, the tension in the venous blood, and the time spent in the capillaries of the lungs. The first of these factors, as shown in this paper, would tend to keep down the tension in arterial blood during the work, the last would tend to raise it by giving less time to establish the resting equilibrium of tensions before the blood leaves the pulmonary capillaries; with regard to the second factor, the tension of carbon dioxid in the venous blood during muscular activity, accurate information is apparently not available. Chauveau and Kaufmann,⁷ Sczelkow,⁸ and Hill and Nabarro,⁹ agree in finding an increase of the volume percentage of carbon dioxid gained by the blood passing through a working as compared with a resting muscle, despite the increase of blood flow through the muscle. This would of course by itself lead to an increase of the carbon dioxid of the caval blood; but muscular activity also involves a more rapid flow through the skin without change in the intensity of the metabolic processes in that organ. This would lower the tension of carbon dioxid in the blood of the cutaneous veins which might counteract the increased tension of that from the muscles. It is obviously impossible to determine *a priori* what will be the final result upon the tension of this gas in caval blood, so that we are left in the dark as to the carbon dioxid tension of the arterial blood during muscular activity, although it is probable that it is somewhat above that of the alveoli; at any rate it is not lower than this, and probably does not, except in the case of very severe and prolonged exercise, depart very far from the normal tension. During vigorous muscular work, then, the respiratory centre is being stimulated by the muscular metabolites and by an almost if not quite normal tension of carbon dioxid in the centre.

⁷ CHAUVEAU and KAUFMANN: *Comptes rendus*, 1886, ciii, pp. 974, 1057, 1153.

⁸ SCZELKOW: *Sitzungsberichte der k. Akademie der Wissenschaften, Wien*, 1862, xlv, p. 171.

⁹ HILL and NABARRO: *Journal of physiology*, 1895, xviii, p. 218.

With the cessation of the work and the diminished production of carbon dioxid in the muscles, the amount of this gas carried to the lungs in the venous blood is lessened at the same time that the stimulus to the centre from the muscular katabolites continues in full force. The overventilation of the lungs discharges carbon dioxid from the alveoli faster than the gas is received from the blood, and a marked fall of alveolar tension results. It would seem that during this period there must also be a decided fall in the tension of carbon dioxid in the arterial blood, since everything which now transpires — the fall of alveolar CO_2 tension and the lessened velocity of blood through the lungs — must contribute toward this result. If the carbon dioxid tension of the arterial blood does thus fall, there should be a lessened tension of this gas in the respiratory centre and hence a diminution of the total stimulus to its activity; there should be, as there actually is, a rapid diminution in the ventilation of the lungs during the first few minutes following the work, and this will continue until the establishment of an equilibrium between the amount of carbon dioxid returned to the lungs by the blood and the amount discharged from the body by the breathing movements. The rapid return of the breathing movements toward normal after work is therefore the result of this washing out of carbon dioxid from the blood. At this time the muscular katabolites will be relatively more important and the carbon dioxid relatively less important in the stimulation of the centre than during the work. This condition is indicated in the relatively long persistence of the low alveolar carbon dioxid tension and the high oxygen tension. With the gradual removal of the katabolite stimulus, the total stimulation of the centre diminishes, the overventilation lessens, carbon dioxid increases in the alveoli and in the arterial blood, and finally after an hour or more the resting condition, in which carbon dioxid is the sole stimulus, is restored.

The failure of Miss Fitzgerald (*loc. cit.*) to get any change of alveolar carbon dioxid tension after a bicycle ride of nine miles in fifty-three minutes (= 10 miles an hour), if we are to accept the results of but one observation as of general application, is of some interest in this connection. From Leo Zuntz's¹⁰ determinations of the carbon dioxid eliminated in walking and bicycle riding we may safely estimate the

¹⁰ L. ZUNTZ: Untersuchungen über den Gaswechsel und Energieumsatz des Radfahrers, Berlin, 1899.

carbon dioxid production in her experiment as twice as great as in my walk experiments, but not so great as in my trot experiments. The fact that she obtained the same values as the normal indicates one of two things. Either the intensity of the work was such that it falls between the values for our walk and trot experiments, where the alveolar carbon dioxid tension, falling with increasing intensity of work, establishes the equilibrium point at about the normal figure; or else it may be that such bicycle riding so distributes the total muscular work over so many large muscles, while the rhythmicity of their work maintains ideal circulatory conditions in them, that there is very complete oxidation with the formation of only a negligible amount of intermediate katabolites. The hyperpnea in such case would be due entirely to the carbon dioxid stimulus, as in the case of the walk, and unless the samples were taken within a minute or two of the cessation of the work, time might have been given to return to normal.

We may also remark that the well-established fact of the progressive fall of alveolar CO_2 tension with the continuance of hard work — which we explain as due to the accumulation in the blood of muscular katabolites — probably accounts for the return toward normal observed by Pembrey and Cook with the advent of second wind. Second wind, after all, merely marks the completion of the adjustment of the organism as a whole, and especially the circulation and respiration, to the given intensity of muscular activity. In work of the kind used by Pembrey and Cook we may suppose that several minutes are required for this adjustment and that by this time overventilation of the lungs has begun. The CO_2 tension has begun to go down, and it may fall to the normal or below normal. Second wind is not simply the re-establishment of the resting CO_2 tension, although Pembrey's work does indicate that the respiratory distress which comes on at the beginning of hard work suddenly entered upon at its full intensity may well be due to the excessive CO_2 tension in the arterial blood.

This is not the place to discuss the chemical nature of the muscular metabolites, whether lactic acid, or lactates, or some other unknown substances; or whether they act only by diminishing the titration alkalinity of the blood or in other ways. The results of my experiments throw little or no light on these questions, so that their discussion here would be out of place. It is most interesting to observe, however, the general parallelism between the duration of the period of secondary

low CO_2 tension and the time during which Ryffel has shown that lactates can be demonstrated in the blood after hard muscular work.

Finally, it is worthy of remark that this secondary lowering of CO_2 tension is unaccompanied by any of the concomitant symptoms of apnea or acapnia. Does the katabolite stimulus take the place, with other centres of the usual carbon dioxid stimulus, as it seems to do with the respiratory centre? We can only raise this interesting and important question.

SUMMARY.

1. An improvement is described in Haldane's method of collecting samples of alveolar air, which, it is believed, makes for greater accuracy.

2. Moderate muscular work (*e. g.*, a walk of four miles an hour) shows a slightly increased alveolar tension of carbon dioxid and of oxygen. The increase of lung ventilation is attributed solely to the increase of the carbon dioxid stimulus to the respiratory centre. This result is in agreement with the work of Douglas and Haldane, who also show that after this work there is no secondary fall of alveolar CO_2 tension.

3. With greater intensity of work (in which the subject "warms up" gradually to the maximum intensity and so avoids being winded in the earlier stages) the alveolar air immediately afterwards shows a fall of carbon dioxid tension and a rise of oxygen tension. These changes increase with the intensity and the duration of the work, a fall of 11 mm. being noted after a hard run of twenty minutes, although it does not usually exceed 5 mm. after a hard run of ten minutes. In none of our experiments was the carbon dioxid tension after such work higher than normal, and reasons are given for regarding this result in the work of previous investigators as a secondary effect of interference with the free working of skeletal and muscular mechanisms of respiration, or else to errors in taking the sample of alveolar air.

4. After the cessation of hard work there is a rapid fall of alveolar CO_2 tension to 26 mm. (normal = 37) and an even more striking rise of oxygen tension to 112-125 mm. This condition persists with undiminished intensity for twenty to thirty minutes and then gradually passes away.

5. An attempt is made to explain all these facts as due to the entrance into the blood of a second respiratory stimulus in the form of some muscular katabolites which are only slowly excreted. Until they are excreted there is present what we have called an overventilation of the lungs.

6. This overventilation is of use to the organism during the work in that the changes in alveolar tensions favor the more rapid diffusion of gases into and out of the blood at the time when the increased speed of the pulmonary circulation gives less time for proper aeration of the blood in the capillaries of the lungs.

THE RELATIVE TOXICITY OF DOG'S NORMAL AND HYPERTROPHIED THYROIDS TO ANIMALS SUSCEPTIBLE TO THYROID FEEDING.

By O. O. STOLAND.

[From the Hull Physiological Laboratory of the University of Chicago and the Physiological Laboratory of the University of South Dakota.]

THE recent work from this laboratory by Carlson and his pupils on thyroid feeding in a number of avian and mammalian species brought out the fact that rats, mice, guinea pigs, rabbits, and pigeons are relatively susceptible to thyroid administration, and that this toxic action is within limits specific for the thyroid. The present experiments were undertaken at Professor Carlson's suggestion as a further test of this specific toxicity, as well as with the hope of throwing further light on the nature of thyroid hyperplasia or goitre prevalent in dogs in the region of the Great Lakes. The studies of Marine¹ and his co-workers and of Carlson and Woelfel² on these dog goitres have brought out the fact that the hypertrophied glands contain a smaller percentage of iodine than do the normal glands. While it is true that the relation of the iodine to the thyroid secretion is still an open question, there seems to be no doubt of the parallelism between the percentage of iodine and the degree of action of the thyroid in certain physiological processes.³ If the toxic action of thyroid administration in certain species is specific for the physiologically active substance in the thyroid, and if the substance is quantitatively related to the iodine in the gland, we should expect that the dog's normal thyroid would be more toxic than the goitrous gland. This proved to be the case.

¹ MARINE and WILLIAMS: Archives of internal medicine, 1908, i, p. 358; MARINE and LENHART: Archives of internal medicine, 1909, iii, p. 66; iv, p. 440.

² CARLSON and WOELFEL: This journal, 1910, xxvi, p. 52.

³ HUNT and SEIDELL: Bulletin Hygienic Laboratory, Washington, 1909, no. 47; 1910, no. 69.

The material for feeding was obtained at the Chicago dog pound, where dogs are killed in large numbers. The glands from these dogs range in size from the small normal glands to the greatly hypertrophied glands, so that it is impossible by direct inspection to draw a sharp line between what may be called "normal" and goitre thyroids. In selecting the glands the smaller ones were called "normal" and the

TABLE I.

IODINE CONTENT OF THE NINE LOTS OF DESICCATED "NORMAL" AND HYPERTROPHIED DOG'S THYROIDS USED IN THE FEEDING.

Thyroid.	Number of glands.	Average weight.		Iodine, per gram of dried glands.		
		Fresh.	Dried.	Maximum.	Minimum.	Average.
		gm.	gm.	mg.	mg.	mg.
"Normal"	587	4	0.68	0.847	0.216	0.440
"Hypertrophied"	216	20	3.70	0.350	0.030	0.233

remaining larger ones were called hypertrophied. The cystic glands were eliminated. In this way it is certain that the thyroids of the hypertrophied lot were greatly hypertrophied, while the "normal" lot was made up of the normal and the slightly hypertrophied glands. According to Carlson and Woelfel,⁴ at least 50 per cent of the dogs in Chicago have distinct enlargement of the thyroid glands. The per cent is probably even larger, as not over 25 per cent of the dogs killed at the pound have normal glands, as determined by direct inspection. The glands were dried, desiccated, and powdered in the usual way and fed in gelatine capsules to rabbits, guinea pigs, and pigeons, and mixed with cracker dust for the rats.

IODINE CONTENT OF THE DOG'S THYROIDS.

Iodine determinations were made on the desiccated thyroid material, as shown in Table I. As might be expected, the contents per gram of dry thyroid was quite constant, since the tests were made from material consisting of a mixture of a large number of glands. The tests were made according to the Baumann-Oswald method. Carlson and Woel-

⁴ CARLSON and WOELFEL: *Loc. cit.*

fel found a great individual variation in the iodine content of the dog's thyroids, but for the "normal" glands the iodine content was greater than for the hypertrophied glands in accordance with the pre-

TABLE II.

Rats fed 0.33 gm. "normal" dog's thyroids per day.				Rats fed 0.33 gm. hypertrophied dog's thyroids per day.			
No. of rats, 18.	Feeding time.	Weight.		No. of rats, 18.	Feeding time.	Weight.	
		Beginning.	At death.			Beginning.	At death.
Aver. . .	days. 19.5	gm. 134.6	gm. 104	Aver. . .	days. 33.6	gm. 102	gm. 83.6

vious results of Marine. The average of my determinations shows that the amount of iodine per gram of dried "normal" glands is about twice as great as that for the hypertrophied glands.

RESULTS OF THE FEEDING.

Tables II, III, IV, and V show the amount of desiccated thyroid fed each animal per day, feeding time, and the weight at the beginning

TABLE III.

Guinea pigs fed 0.33 gm. "normal" dog's thyroid per day.				Guinea pigs fed 0.33 gm. hypertrophied dogs thyroid per day			
No. of guinea pigs, 12.	Feeding time.	Weight.		No. of guinea pigs, 12.	Feeding time.	Weight.	
		Beginning.	At death.			Beginning.	At end.
Aver. . .	days. 10.9	gm. 479	gm. 318.7	Aver. . .	days. 22.7	gm. 472	gm. 317

of the experiment and at the end of the experiment or at death, if feeding was carried on until death. Rats and guinea pigs were found to be more susceptible to the feeding than were rabbits and pigeons, but *in all cases the animals die earlier from feeding of "normal" than from feeding of hypertrophied thyroids.* For rats and guinea pigs the feeding time is about twice as long for those fed on goitre glands as those fed on "normal" glands. The toxicity, then, is inversely proportional

to the iodine content, at least in these cases. In all cases the animals lost weight very noticeably the first few days, but, if they survived this first loss, they remained nearly constant in weight until a few days

TABLE IV.

Pigeons fed 0.33 gm. thyroid per day for seventeen days; 0.66 gm. per day for forty-five days; 1 gm. per day for twenty-eight days.							
"Normal" dog's thyroid.				Hypertrophied dog's thyroid.			
No. of pigeons, 6.	Feeding time.	Weight.		No. of pigeons, 6.	Feeding time.	Weight.	
		Beginning.	At death.			Beginning.	At end.
Aver. . .	days. 108.6	gm. 316.6	gm. 200.8	Aver. . .	days. 134+	gm. 366.6	gm. 279

before death. Most of the pigeons and all the rabbits fed on hypertrophied material remained at fairly constant weight after the initial loss, and survived so long that the experiment was discontinued.

That the first loss of weight in the case of the rats is due in part to the handling and mechanical effect of feeding and weighing seems to

TABLE V.

Rabbits fed 0.33 gm. dog's thyroid per day for nineteen days; 0.5 gm. per day for thirty days; 1 gm. per day to end of experiment.							
"Normal."				Hypertrophied.			
No. of rabbits, 3.	Feeding time.	Weight.		No. of rabbits, 3.	Feeding time.	Weight.	
		Beginning.	At death.			Beginning.	At end.
Aver. . .	days. 76	kgm. 2.10	kgm. 1.25	Aver. . .	days. 101+	kgm. 2.30	kgm. 2.01

be shown by a control experiment on 12 rats. The rats in this group of experiments were weighed every day and all of them lost weight, but the thyroid feeding was omitted until their weight had nearly reached that at the beginning. The thyroid feeding was then started and continued for fifteen days without the loss of a single animal. Six of the rats were young animals and even increased in weight during the thyroid feeding. The older rats fed on "normal" glands lost

some weight during the feeding, but those fed on goitre thyroids remained practically constant in weight. It was very unfortunate that the experiment was brought to an untimely end by the death of the rats during the shipment from Chicago to South Dakota.

CONCLUSIONS.

1. In the case of animals very susceptible to thyroid feeding, dog's normal thyroids are more toxic than the hypertrophied thyroids.

2. As the hypertrophied thyroid contains a smaller percentage of iodine, the degree of toxicity of the dog's thyroids, as determined by this test, is, on the whole, inversely proportional to their iodine content. The toxic material is more concentrated in the normal than in the hypertrophied glands.

3. In the case of the rats the loss of weight during the first few days of thyroid feeding is partly due to the handling in feeding and weighing.

I am greatly indebted to Dr. A. J. Carlson for very valuable suggestions and criticisms, as well as for thyroid material sent me for the part of the experiments conducted in the laboratory at South Dakota.

A NOTE ON THE EFFECTS OF INTRAVENOUS INJECTIONS OF THYROID PRESSURE LIQUID IN DOGS AND CATS.

By G. H. CALDWELL.

[From the Hull Physiological Laboratory of the University of Chicago.]

THE results of experimental work so far reported from this laboratory seem to show that dogs and cats exhibit a remarkable resistance to the internal secretion of the thyroid glands. Large amounts of fresh as well as desiccated gland have been fed to dogs daily for months without producing effects which can clearly be attributed to the specific nature of the substance fed. To supplement these results a series of tests were carried out, at Professor Carlson's suggestion, to determine whether the dog is equally resistant to thyroid pressure liquid introduced directly into the blood. Fresh thyroid material from dogs, removed and used immediately after the death of the animals, was chosen for this work. Only those glands of approximately normal size were taken and, after being washed and freed from other tissues, they were chopped up fine, ground with sand, and mixed with Fuller's earth until of about the consistency of putty. This material was then placed in the Buchner press and subjected to a pressure of approximately three hundred atmospheres until practically free from fluid. The liquid thus expressed was used for all of the experiments.

This pressure liquid was injected intravenously by puncture of the saphenous vein through the skin and hence without anæsthesia. In most cases the injections were made as soon as the pressing was completed, and in all cases the material was kept on ice so as to minimize the bacterial growth. While scrupulous cleanliness was observed it was impossible to sterilize completely either the material itself or the apparatus used; hence the pressure liquid as finally obtained was not sterile. But, as the injections did not cause any apparent infection, the natural immunity of the dogs evidently took care of the bacteria thus introduced directly into the blood.

RESULTS.

- Dog I.* — Weight, 7 K. On June 25, July 7, and July 11 intravenous injections of the pressure liquid of 30, 50, and 40 dogs' thyroids respectively. No change in weight or change in pulse after the immediate effects of the injection. Dog died within ten hours after the last injection.
- Dog II.* — Weight, 9 K. On June 29 intravenous injection of the pressure liquid of 15 dogs' thyroids. No effects on body weight or pulse.
- Dog III.* — Weight, 4 K. On July 1 intravenous injection of the pressure liquid of 80 dogs' thyroids. No effect on body weight or pulse.
- Dog IV.* — Weight, 6 K. On July 13, 15, 18, 22, and 25 intravenous injections of 25, 66, 40, 56, and 40 dogs' thyroids respectively. No effect on body weight or pulse.

The data on Dog V are presented in tabular form in Table I.

- Cat I.* — August 22. Intravenous injection of the pressure liquid of 20 dogs' thyroids. Only temporary effects.
- Cat II.* — August 26. Intravenous injection of pressure liquid of 35 dogs' thyroids. Only temporary effects.
- Rabbit I.* — August 24. Intravenous injection of the pressure liquid of 12 dogs' thyroids. Dyspnea, palpitation, tremors, and death within two minutes.
- Rabbit II.* — August 26. Intravenous injection of the pressure liquid of 16 dogs' thyroids. Death within two minutes with same symptoms as I.
- Rabbit III.* — August 26. Intravenous injection of the pressure liquid of 10 dogs' thyroids. Death within five minutes with same symptoms as I.

In most instances the injections in the dogs and cats were followed by temporary depression, increase in rate of heart beat, vomiting, defecation, and micturition. Within fifteen to sixty minutes the condition of the animals as judged by external signs had returned to practically normal. After the third injection Dog I collapsed, the heart beat became slow and weak, and the respiration was dyspneic, with extreme prostration. A half-hour later the animal had improved greatly, but it died during the night. As none of the other dogs reacted in this way, it is probable that death in this case was due to anaphylaxis.

Dogs II and III received only one injection each, the results being negative in each case, except for the temporary effects. Dog IV received five injections and recovered well from each, not even losing

TABLE I.

Date, 1911.	Wt. K.	Pulse.		No. gld. inj.	No. c.c. inj.	I. per gl. mg.	I. per gl. in liq. mg.	Total i. inj. mg.	Immed. effects
		Bef.	Aft.						
July 26	5.7	120
" 27	5.6	124
" 28	5.5	130
" 29	6	125	125	75	150	?	.18	13.5	Vomiting.
Aug. 1	5.7	40	75	.3	.18	7.2	Defecation.
" 2	5.5	150	150	40	60	.15	.12	4.8	"
" 8	5.03	120	130	40	65	"
" 10	4.7	134	200	40	54	?	.08	3.2	"
" 15	4.55	140	160	35	46	.15	.09	4.05	Negative.
...	...	110	140
" 17	4.74	156	160	42	Vomiting, etc.
" 19	4.8	42	57	.19	.06	2.42	" - severe.
" 21	160
" 26	5.35	...	160

weight. Dog V received eight injections, reacting after each injection about as described above. In this dog there was progressive loss of weight with gastric and intestinal disturbances and apparently a slightly increased pulse rate. There was rapid recovery of body weight following the discontinuance of the injections.

The cats exhibited practically the same symptoms as the dogs. The rabbits showed in each case marked dyspnea, tremors, and depression, resulting in death almost immediately, apparently from paralysis of the heart. Rabbit III received a smaller dose than the other two and did not succumb quite so rapidly.

It was not until the work with Dog V was begun that the iodine content of the material was determined. After the glands were chopped up fine they were weighed, and a known quantity, representing one average gland taken from various parts of the mixture, was saved and the iodine determined. Obviously this procedure was not accurate, as the mixing could not be perfect, but served for purposes of comparison. In the case of the pressure liquid, however, the whole amount was mixed and measured. Then an amount representing five glands was used for iodine determination by the same method.

The study of the changes in the heart's action was very unsatisfactory because of the great irregularity of the heart rate in dogs and its susceptibility to nervous influences such as fear, excitement, vomiting, etc. However, our results gave no reason for supposing that the heart was materially influenced by the injections, as the average rate after recovery from the temporary effects was not different from that before injection, except possibly in the case of Dog V. There were no changes which might not be satisfactorily accounted for by the conditions present. The temporary changes were undoubtedly due to the foreign proteins introduced into the blood causing a fall in blood pressure, and, secondarily, to the gastro-intestinal disturbances.

SUMMARY.

The results of this work furnish additional evidence of the high resistance of dogs and cats to the specific thyroid substance. The fact that cats show as great resistance as dogs do to thyroid material from dogs seems to show that the resistance in the latter was not due

solely to the source of the material. The fatal results in the rabbits may be explained by a high susceptibility on their part to an increase of thyroid substance in the blood, or to the specific effect of the foreign proteins. In general these three species exhibit the same relative resistance to thyroid pressure liquid introduced intravenously and to thyroid given by mouth.

THE EFFECTS OF BLOOD TRANSFUSION IN PARATHYROID TETANY.

By CLARA JACOBSON.

[From the Hull Physiological Laboratory of the University of Chicago.]

THE work here presented was undertaken at Dr. Carlson's suggestion in an endeavor to determine whether the parathyroids act by removing or changing some toxic substance present in the blood or by means of an internal secretion. The severe nervous symptoms following parathyroidectomy have suggested the presence of a circulating toxin. Such substances must come from body or food metabolism. They may be present normally, but reach a dangerous concentration only when the "detoxicating" organs are deficient or absent. On the other hand, they may be abnormal metabolites, either qualitatively or quantitatively, resulting from the lack of proper stimulation or regulation by the internal secretion. Most of the experimental results can be explained equally well on either of these two hypotheses. The strongest evidence in favor of the internal secretion theory has been the apparently specific action of extracts of thyroid and parathyroid glands and of the nucleoprotein isolated from the latter by Beebe, in suppressing the symptoms of parathyroidectomy. But we have found that tetany is suppressed by measures lowering the blood pressure. This suggested that these tissue extracts may also act in this way, particularly in view of the fact that tissue extracts in general, parathyroid included, are known to lower the blood pressure. This is especially the case when the pressure is higher than normal before the injections. Thus the suppression of symptoms by parathyroid extracts, as experiments have been carried out, may not be a specific action.

If the gland does act by means of an internal secretion, it should be possible to isolate this substance from normal blood providing the proper methods were used. Transfusing normal dog's blood into

dogs completely thyroidectomized should also demonstrate the physiological action of this internal secretion. The fact that a very small remnant of parathyroid tissue will suffice to prevent the development of symptoms speaks in favor of such an experiment. Injections or transfusion of large quantities of normal blood ought to markedly decrease or entirely prevent the appearance of symptoms.

LITERATURE.

Toxemia in parathyroid tetany.¹—As above stated, the symptoms of parathyroidectomy have suggested the presence of a circulating toxin. They have been compared with symptoms of certain convulsive poisons, as picrotoxin and ammonia, and attempts have been made to establish a relationship to organic toxemias as infantile tetany, gastric tetany, uremia, and eclampsia. Pregnancy near term augments the parathyroid tetany symptoms. It is claimed that the urine of animals in tetany gives rise to toxic symptoms when injected into other animals. Chemical analysis of the urine has shown increased ammonia excretion, but the extent to which this may have been due to anorexia and other conditions possibly not associated with the

¹ (Toxemias) CHVOSTEK: Wiener klinische Wochenschrift, 1905, xviii, p. 969; ERDHEIM: Mitteilungen aus den Grenzgebieten der Medizin und Chirurgie, 1906, xvi, p. 632; (Infantile Tetany) PINELES: Jahrbuch für Kinderheilkunde, 1907, lxvi, p. 665; BING: Zentralblatt für die gesammte Physiologie und Pathologie des Stoffwechsels, 1908, iii, p. 52; YANASE: Jahrbuch für Kinderheilkunde und physische Erziehung, 1908, lxvii, p. 57; GLASERFELD: Berliner klinische Wochenschrift, 1909, xlvi, p. 112; (Uremia) DUTTO and MONACO: Archives italiennes de biologie, 1895, xxiv, p. 196; (Epilepsy) CLAUDE and SCHMERGELD: Comptes rendus de la Société de Biologie, 1908, lxv, pp. 80 and 138; (Eclampsia) VASSALE: Archives italiennes de biologie, 1898, xxx, p. 49; *Ibid.*, 1903, xliii, p. 177; *Ibid.*, 1906, xlvi, p. 143; CENI: Archives italiennes de biologie, 1902, xlii, p. 420; LORTAT: Comptes rendus de la Société de Biologie, 1904, lvi, p. 61; FROMMER: Monatschrift für Geburtshilfe und Gynäkologie, 1906, xxiv, p. 748; (Toxicity of the Urine) MASOIN: Comptes rendus de la Société de Biologie, 1894, xlvi, p. 105; (Ammonia in the Blood and Urine) CORONEDI and LUZZATO: Archives italiennes de biologie, 1907, lxvii, p. 286; MACCALLUM and VOEGTLIN: Journal of experimental medicine, 1909, xi, p. 118; GREENWALD: this Journal, 1911, xxviii, p. 103; COOKE: Journal of experimental medicine, 1911, xlii, p. 439; CARLSON and JACOBSON: this Journal, 1911, xxviii, p. 133; (Blood) BALDI: Archives italiennes de biologie, 1899, xxxi, p. 281; CENI and BESTA: *Ibid.*, 1904, xlii, p. 455.

toxemia is not known. Dutto and Monaco have observed a diminished azote excretion. On the other hand, the injection of blood from animals in tetany into animals parathyroidectomized but not showing symptoms appears without effect. The reported results of analyses of the ammonia content of the blood are conflicting.

Histological changes in organs,² following complete thyroidectomy, are considered in general by Kishi and by Bensen. Degenerative changes have been noted in the brain and spinal cord, by some in the fresh gross specimens, by others through special fixing and staining methods. Defective osteogenesis has been observed. Degenerative changes have also been noted in the kidney, digestive tract, and liver, but these are not always well marked. The physiological significance of these changes is not known.

METHODS.

In this series of experiments small dogs weighing about 4 to 5 kilos were used. Complete thyroidectomy was performed, and twelve to eighteen hours later normal defibrinated blood was injected intravenously, and this injection repeated every twenty-four hours. In a few cases, as indicated in the table, blood injections were made twice daily. About 15 to 20 c.c. of blood per kilo body weight were used daily in each case. The blood was obtained directly from the heart of normal dogs under relatively aseptic conditions and without anæsthesia. In two cases the parathyroidectomized dogs showed mild symptoms, but lived two weeks, and blood injections were then stopped to see if acute nervous symptoms would appear. Two other dogs showed no symptoms whatsoever, and injections were stopped at the end of one week. No medication, as for instance intra-

² BABONNEIX and HARVIER: *Comptes rendus de la Société de Biologie*, 1894, xlv, p. 105; PISENTI: *Archives italiennes de biologie*, 1894, xxi, p. 15; ROUXEAU: *Archives de physiologie*, 1897, ix, p. 136; BENSEN: *Virchow's Archiv für pathologische Anatomie, Physiologie, und klinische Medizin*, 1902, clxx, p. 229; KISHI: *Ibid.*, 1904, clxxvi, p. 260; ALQUIER and THEUVENY: *Comptes rendus de la Société de Biologie*, 1907, lxii, p. 963; MANCA: *Archives italiennes de biologie*, 1907, xlvii, p. 332; MASSAGLIA: *Ibid.*, 1908, l, p. 367; MOREL: *Comptes rendus de la Société de Biologie*, 1909, lxvii, p. 780; *Ibid.*, 1910, lxviii, p. 163; DELITALA: *Archives italiennes de biologie*, 1908, xlix, p. 109; ERDHEIM: *Frankfurter Zeitschrift für Pathologie*, 1911, vii, p. 175.

venous injections of calcium lactate, was given to suppress the tetany at any time. When, as we have frequently observed, the temperature rose above 102° in tetany, cold baths were given until the temperature returned to 101° or 100° . The results obtained from other animals thyroidectomized and observed in this laboratory previously, were taken as controls. Post-mortem examinations were made in all cases, special attention being directed to the condition of the alimentary tract.

The method of direct transfusion of normal blood into the thyroidectomized dogs in place of intravenous injections was considered, but not adopted because of the obvious difficulty of repeating this operation daily over a sufficiently long period of time to be conclusive. Danger of infection would be increased and the effects of repeated anæsthetics would be a disturbing factor. On the whole, then, this method does not seem as practical as the one followed.

There are, however, two main criticisms on the present method to be noted. We do not know the properties of the internal secretion we may be dealing with. It is possible that in the process of defibrination we may destroy or remove the internal secretion, or the secretion may be unstable and lose some of its activity in the interval between the drawing of the blood from one dog, its defibrination and unavoidable cooling, and its subsequent injection into the other dog. Secondly, we have the possible injurious action of daily injections of large quantities of foreign blood. In answer to the second objection, however, we have the record of two dogs which failed to show any symptoms of the parathyroidectomy. These dogs received daily injections of the normal blood for one week without showing any change in their general good health.

RESULTS.

The results are summarized in Table I. The average survival of the 14 dogs under this treatment was ten and a half days. Four dogs lived thirteen days or longer. Dog 5 suffered from a hemorrhage into the wound area and was subjected to a second operation, May 17, after which no further blood injections were made. Acute tetany symptoms developed the day following. Death occurred during the night, so that the condition at the time of death is unknown. Indications were that death was caused by hemorrhage possibly resulting

TABLE I.

SUMMARY OF EXPERIMENTS ON FOURTEEN PARATHYROIDECTOMIZED DOGS, WITH DAILY INJECTIONS (INTRAVENOUSLY) OF 15-20 C.C. NORMAL DEFRIBRINATED BLOOD PER KILO BODY WEIGHT.¹

No.	Wt. K.	Date of operation.	Date of death.	Days survived. ²
1	7.7	Apr. 17	May 1	13½ ³
2	4.0	" 17	" 2	15 ⁴
3	6.0	" 17	Apr. 29	11½ ⁵
4	8.0	" 17	" 20	2½ ⁶
5	6.26	May 3	May 20	16½ ⁷
6	6.5	" 3	" 7	4 ⁸
7	4.44	" 3	" 11	8 ⁹
8	4.6	" 8	" 17	10 ¹⁰
9	3.6	" 8	" 18	9½ ¹¹
10	4.0	" 23	" 31	7½ ¹²
11	5.3	" 23	" 31	7½ ¹³
12	5.4	June 19	June 25	6 ¹⁴
13	7	" 19	July 3	14 ¹⁵
14	4	July 3	" 13	10 ¹⁶

¹ Dogs Nos. 5 to 11 inclusive received two injections daily.

² Average period of survival of the fourteen dogs transfused with blood ten and one-half days.

³ Tremors. Rigidity of limbs when walking. Marked depression.

⁴ Tremors on 18th. Convulsions first four days, also second day after cessation of blood injections, May 1.

⁵ Tremors on 19th, 20th, 21st, 22d. Convulsions 21st. Restlessness and rapid respiration following days.

⁶ Slight tremors.

⁷ Depression and tremors 14th. No blood injection on or after the 18th. Typical tetanic convulsions 18th.

⁸ Tremors 4th and 5th. Slight tremors the 6th.

⁹ Convulsion spasms incident to handling on 5th. Hemorrhage in wound. Anesthetized on 11th to find source of hemorrhage. Death in severe tetanic spasms at this time.

¹⁰ Tremors 15th and 16th. Convulsions 17th and 18th.

¹¹ Intermittent tremors almost every day.

¹² Tremors 26th and 29th. Groaning. Post-mortem: lobar pneumonia.

¹³ Depression but no tremors. Skin infection in site of injections. Mange.

¹⁴ Tremors 21st, 22d, 23d, 25th.

¹⁵ Convulsions 28th.

¹⁶ Tremors and tetany on 8th.

from an increased blood pressure in a tetanic convulsion. In the other dogs progressive depression rather than acute nervous trouble seemed to be the rule. As an illustration of the general results we may cite the following abstract of the protocol in one experiment, Dog No. 2.

- April 17*, 3.15 P. M. Thyroidectomized; glands small.
- April 18*, 8.30 A. M. Strong tremors in legs; dyspnoea.
9.30 A. M. Injection of 90 c.c. of normal blood; symptoms remain the same.
11.00 A. M. Possible slight diminution in symptoms.
1.00 P. M. Depression; slight tremors in shoulder muscles.
2.05 P. M. No tremors, general appearance much brighter.
- April 19*, 9.00 A. M. Depression, but no tremors. Injection of 50 c.c. normal blood.
- April 20*, 8.30 A. M. Depression, but no tremors. Injection of 120 c.c. normal blood.
2.00 P. M. Strong tremors, rigid limb extension, cannot walk when put on his feet, respiration rapid, 100 c.c. blood injected.
- April 20*, 3.30 P. M. Blood drawn from femoral artery; respiration good; tremors diminished.
4.00 P. M. Tremors entirely gone.
- April 21*, 9.30 A. M. Slight tremors; respiration normal; 100 c.c. normal blood injected.
- April 22*, 9.30 A. M. No tremors; respiration normal; depression, 75 c.c. blood injected.
- April 23*, 9.30 A. M. No tremors; respiration normal; depression, 75 c.c. blood injected.
- April 24*, 9.30 A. M. No tremors; respiration normal; depression, 75 c.c. blood injected.
- April 24*, 3.00 P. M. No tremors; diaphragm contractions synchronous with heart beats; 50 c.c. blood injected.
- April 25*, 9.30 A. M. Slight tremors; diaphragm contractions, depression, 75 c.c. blood injected.
- April 26*, 9.30 A. M. Depression, tremors, and snapping of jaws followed by violent fits of sneezing; these disappear within five minutes, 75 c.c. blood injected.
- April 27*, 8.30 A. M. Like yesterday. 75 c.c. blood injected.
- April 28*, 8.30 A. M. Like yesterday. Sneezes less, less depressed, 75 c.c. blood injected.

April 29, 8.30 A. M. No tremors, less depressed, diarrhea, 75 c.c. blood injected.

April 30, 8.30 A. M. No tremors, less depressed, diarrhea, 75 c.c. blood injected.

May 1, 8.30 A. M. No tremors, less depressed, diarrhea, no blood injected.

May 2, 8.00 A. M. Breathing at times labored, transient tremors, vomiting, nose infected.

12.00 M. Convulsions, tonic contractions.

12.15 P. M. Tetanic convulsions. Bled to death from carotids.

Post-mortem: Stomach and intestines in unusually good condition, only slightly hemorrhagic in duodenum and at pylorus. One small ulcer in stomach.

These experiments show that injections of normal blood neither absolutely prevent the onset of symptoms nor act promptly, if at all, in suppressing them, but the lives of the animals appear to be prolonged. The injections seem to have some effect in delaying or relieving the acute tetany symptoms, but the more chronic depression persists to a fatal culmination.

The post-mortem findings in the stomach and intestines are similar to those previously described. Ulcers and a hemorrhagic mucosa are just as marked in those examined immediately after death as those examined within twelve hours of death, so that these findings may not be considered as post-mortem changes. Neither can they be attributed to iso-lysin action of foreign blood, for these changes have already been reported as occurring in animals not treated this way. There are differences in the extent and severity of these lesions, just as there are differences in the constancy of other characteristics of parathyroid tetany; for example, salivation, typical of and yet frequently absent in this form of tetany.

DISCUSSION OF THE RESULTS.

The results we have obtained are not so conclusive as we had expected, but so far as they go, they seem to favor the internal secretion hypothesis. From the standpoint of the detoxication theory, these injections may be considered as diluting the blood and increasing

the blood pressure and thus promoting elimination. The injection of large quantities of a concentrated NaCl solution has proved efficient in prolonging the lives of tetany animals by Meltzer and Joseph, but we do not know whether diluting the blood with isotonic salt solution to the same extent that is done by the blood transfusions would prove as efficient. Even in our series of calcium injections the average prolongation of life was not so great as in the transfusion experiments. It is possible that the early introduction of so much foreign blood into an animal already weakened by parathyroidectomy has a depressing effect and thus augments the progressive depression already present. This may prevent the appearance of tetany, and the animal is saved the strain and loss of energy incident to the tetany. However, the two control animals already mentioned received the same quantities of blood yet showed no depression.

If our results are due to the action of a specific internal secretion, this secretion must be present in the blood in very small quantities or else rapidly destroyed or lost under the conditions of our experiments. Since a small fraction of the parathyroid seems to prevent the development of the tetany symptoms, and because of the further fact that in dogs tetany usually does not develop until two or more days after complete parathyroidectomy, one would expect a more marked effect from blood transfusion. It is possible, however, that the delay in the development of tetany after complete thyroidectomy indicates a series of processes necessary to establish the conditions of tetany rather than the persistence of the parathyroid secretion in the blood. It is well known that in exceptional cases tetany may appear within less than twenty-four hours of the parathyroidectomy. These exceptions point to a rapid destruction or elimination of the secretion when once in the blood and lymph. If this interpretation is correct, we cannot look for marked or lasting improvements from blood transfusion in parathyroid tetany.

SUMMARY.

Intravenous injection once or twice daily of defibrinated blood of normal dogs into parathyroidectomized dogs prolongs the life of these animals on the whole, but it has no immediate or marked action

on the tetany and the depression symptoms. These transfusions have no obvious injurious effects on normal animals.

Accepting the internal secretion hypothesis, our results indicate a rapid destruction and elimination or a very slight concentration of the parathyroid secretion in the body fluids.

THE COMPARATIVE TOXICITY OF DIFFERENT ANIMAL TISSUES TO ANIMALS SUSCEPTIBLE TO THYROID FEEDING.

By H. E. FRENCH.

[From the Hull Physiological Laboratory of the University of Chicago.]

THIS study was undertaken, at the suggestion of Professor Carlson, as a part of the research work that is being devoted by him to the thyroid gland. Its object has been to discover whether the toxicity of the commercial desiccated thyroid when given by mouth is specific, or whether similar effects can be produced by other animal tissues prepared and fed in the same way; whether it is due to decomposition products; or whether it is due simply to the great amount of proteid matter ingested by an animal unaccustomed to such a diet.

The literature of the whole subject has been reviewed recently by Professor Carlson.¹ Very little of it applies directly to the question in hand except the work of Cunningham.² In 1898 Cunningham published the results of some extensive experiments carried out two years earlier. He found that extracts of thyroid glands given hypodermically are toxic, but that other tissue extracts given in the same way are also toxic, and he thought that extracts made from stale materials were more toxic than those made from fresh tissues. He reported that absolutely fresh calf's and sheep's thyroids given to four rabbits by mouth in 40 and 50 gm. doses for eighteen days are harmless, while one feeding of the same material kept in the ice box for twenty-four hours is toxic enough to kill the animals in a few hours. He secured exactly the same results with stale meat and extracts of stale meat, but observed that for the most striking effects other material should be ten to fourteen days old, but not putrid. He fed the various commercial thyroid preparations of that time, getting more or less toxic and widely vary-

¹ CARLSON, ROOKS, and MCKIE: this Journal, 1912, xxx, p. 129.

² CUNNINGHAM: Journal of experimental medicine, 1898, iii, p. 147.

ing effects. He found no evidence of toxicity when any of the above-mentioned substances were fed to dogs and cats. Exophthalmus was observed in rabbits, not only after some of the thyroid feeding, but also after toxic meat extracts. Cunningham concluded that absolutely fresh thyroid is not toxic when given by mouth, and that the symptoms of experimental thyroidism are due to decomposition products, and that they are not peculiar to the thyroid. Carlson and his students have been making extensive experiments with thyroid feeding in the last two years. They have found that the commercial desiccated thyroid or the gland dried and pulverized in the laboratory is toxic for many animals. Different genera exhibit great variations in their susceptibility; dogs, cats, foxes, and ducks being the most resistant of all animals fed, rodents and primates the least. The most constant symptoms are emaciation and diarrhoea, with death in convulsions or in depression; no exophthalmus has been observed; post-mortem examinations have shown hyperæmia of the intestines with infiltration of the intestinal mucosa. A few observations indicated that this toxicity is not shared by other tissues.

In the experiments that follow, both fresh and desiccated materials have been used. In the one case various tissues of the sheep—thyroid, brain, liver, spleen, kidney, and skeletal muscle—were prepared in a manner as nearly like that used in the preparation of the commercial desiccated thyroid as the facilities of the laboratory would permit. The tissues were secured fresh, stripped of fat and connective tissue, finely minced, desiccated in small quantities in a drying bath at about 85° C., and ground to fine powders. The powders were fed to certain groups of animals, the commercial preparation was fed to other groups, no tissue at all was given to still others. In the other case what might be roughly called fresh material was used. The term includes, however, absolutely fresh thyroid, stale thyroid, and stale butcher's meat. By absolutely fresh thyroid is meant the thyroids of animals killed on the same day on which the glands were fed—not more than two or three hours elapsing between the slaughter of the one animal and the feeding of the other. The writer went every day to a large sheep-killing house, saw the glands removed from animals slaughtered only a few minutes before, received the warm glands, hastened back to the laboratory, minced them with a clean knife upon a clean board, and fed them at once. By stale thyroid is meant part of the

material above kept in a laboratory ice box for from one to three days. By stale butcher's meat is meant meat such as butchers sell to feed dogs — it was very stale when purchased, and was then kept in the ice box from one to four days; it was never putrid, but it was surely from six to ten days old when used, probably much older.

The animals fed were white rats, guinea pigs, and rabbits, chosen because these animals have been found to be most susceptible to thyroid feeding. They were kept in convenient cages in large cement-floored animal rooms, light, dry, warm, well ventilated, and fairly clean. There was no overcrowding, and there was plenty of dry bedding and good food.

The technique of feeding was not particularly difficult, but it was tedious and required care. Rats were fed by mixing the required dose with cracker dust and water, and placing the mixture before them; this was done in the mornings; other food was given later in the day if the required food had been eaten. Rabbits and guinea pigs were fed the dry material and to some extent also the moist in gelatin capsules. The capsule or a little of the moist material was placed in the animal's mouth, and the rabbit or the guinea pig was then held and watched until the material was either swallowed directly or chewed and swallowed. In every case the efforts persisted until it was certain that the animal had really taken the dose recorded. Feeding was done regularly, except that animals fed on thyroid, becoming markedly sick after five to six feedings, were often given smaller doses or none at all on some days.

Weight, appetite, and other conditions indicating health or disease were carefully observed. Heart and respiration were watched in a general way, but they are always so rapid and variable that little importance can be attached to them; any records made were based upon the comparative rapidity of respiration in groups of animals observed very quietly and at some little distance. Temperatures were recorded for a time, but, seeming to indicate nothing, they were discontinued. Something of a post-mortem examination was made in every case of death, and in a few cases microscopic examinations were made of liver, kidneys, lungs, stomach, and intestines.

In all, some seventy rats, forty-five guinea pigs, thirty rabbits, and two pigeons have been fed. The work was done in six different series, or tines, of from three to eight weeks each. The general health of rats

and rabbits varied somewhat at the different times irrespective of feeding. The chief facts are summarized in Table I.

The results of the feeding of fresh and stale sheep's thyroid and of stale meat may be briefly stated as follows:

FRESH SHEEP'S THYROID.

One rabbit given 20 gm. per day, died on the . . . 10th day.
One rabbit given 50 gm. per day, died on the . . . 7th day.
Three guinea pigs given 10gm. per day, died on the 7th day.

STALE SHEEP'S THYROID.

One rabbit given 20 gm. per day, died on the . . . 5th day.
One rabbit given 40 gm. per day, died on the . . . 3d day.
One rabbit given 50 gm. per day, died on the . . . 2d day.
One guinea pig given 10 gm. per day, died on the . . 8th day.
One guinea pig given 20 gm. per day, died on the . . 3d day.

STALE MEAT.

Two rabbits given 20 gm. per day	} no fatality.
One rabbit given 50 gm. per day	
One guinea pig given 10 gm. per day	

From the table it will be seen that almost every animal fed on thyroid in any form died in a short time; the average time of survival under this feeding was a little less than seven days for twenty-nine animals; the average number of feedings was between five and six. Three rats survived twelve daily feedings of .33 gm., and had to pass out of observation, but they had lost much weight and were taking the typical course; one guinea pig and two rabbits in the same way passed from observation after two or three feedings on larger doses of stale thyroid; no doubt some of these animals died later as a result of the feeding. The clinical courses were wonderfully uniform; there were rapid emaciation, vigorous appetites, and often diarrhoea, followed by a day or two of restlessness, muscular tremor, sometimes convulsions, sometimes coma, sometimes very evident abdominal pain, and death. There seemed to be rapid heart and respiration. There was no exophthalmus. Post-mortem examinations showed in every case a marked hyperæmia of the stomach and intestines, sometimes fluid fecal matter in the colon and rectum; microscopic examination added nothing.

TABLE I.
SUMMARY OF RECORDS OF BODY WEIGHT ON FEEDING DESICCATED THYROID AND OTHER TISSUES.

Material.	Animal.	Dose. gm.	Feeding day.	Weight. gm.	Feeding day.	Weight. gm.	Feeding day.	Weight. gm.	Gain or loss.
Brain	Rats, 4, average weight	.33	1	100	12	106	20	108	Gain.
	Guinea pigs, 4, av. wt.	.50	1	450	11	542	22	540	Gain.
	Rabbit, 1	1.00	1	1275	13	1400	26	1300	Gain.
Muscle	Rats, 4, av. wt.33	12	97	23	93
	Guinea pigs, 4, av. wt. .	.50	1	475	11	525	23	650	Gain.
	Rabbit, 1	1.00	1	2200	13	2400	28	2300	Gain.
Kidney	Rats, 4, av. wt.33	12	87	23	93	Gain.
	Rats, 2, av. wt. . . .	2.00	8	175	22	200	Gain.
	Guinea pigs, 4, av. wt. .	.50	1	400	13	488	22	500	Gain.
Spleen	Rabbits, 2, av. wt. . .	.50	1	1688	12	1950	22	1900	Gain.
	Rats, 4, av. wt.33	1	110	18	110	29	100	Loss.
	Rat, 1	1.00	1	75	16	90	29	80	Gain.
Liver	Rats, 4, av. wt.33	1	94	16	95	29	95	Gain.
	Guinea pigs, 2, av. wt. .	1.00	1	400	20	500	28	595	Gain.
	Rabbit, 1	1.00	1	2000	14	2000	27	1900	Loss.
Thyroid	Rats, 2, av. wt.33	1	175	7	150	12	100 †	Loss.
	Rat, 133	1	80	3	70	14	60 †	Loss.
	Guinea pigs, 2, av. wt. .	.50	1	700	6	495	9	350 †	Loss.

Most of the animals fed on other tissues did not die, and not only did they survive, but many gained in weight, and with hardly an exception they seemed perfectly well at the end of the experiment. The gain in weight is no doubt to be accounted for by growth, and by the better care given to the animals while under observation. No death and no unfavorable symptoms can be attributed to the feeding. In August, 1910, one rabbit on brain feeding and two on muscle feeding died with symptoms of emaciation, infected cuts or bites on lips, noses, and other parts of the body, and discharges of pus from nose, mouth, and eyes; post-mortem findings were those of inflammation of the respiratory organs. But unfed animals in the same room also died with the same symptoms and findings; and rabbits at the same time in another room fed on kidney, and all rabbits fed at other times on any tissue but thyroid remained perfectly well. Some of the rats fed from November 28, 1910, to February 3, 1911, died with symptoms of loss of weight and warty excrescences about heads, tails, and genitals; the post-mortem findings were those of gastro-enteritis, in one case there was an encysted parasitic worm in the liver. Some of the rats also died on two very cold nights. But, as with the rabbits in August, control rats seemed to be as badly affected as those fed, and died in the same proportions, and with the same symptoms and findings; and rats at other times with one exception remained perfectly well. One rat fed on 2 gm. of kidney died in July, 1911, with indefinite symptoms and findings, but two others on the same feeding remained perfectly well, gained an average of 25 gm. in weight in the three weeks they were under observation, and then survived the rest of the summer.

Thyroid was fatal in every form: commercial, desiccated in the laboratory, absolutely fresh, and stale. Three guinea pigs and two rabbits on feedings of absolutely fresh thyroid perished in time, and with symptoms exactly the same as those fed on other forms. Stale thyroid killed one rabbit in five days and one guinea pig in eight days, but the symptoms were the same. Stale thyroid in the two cases just mentioned, and in the case of one guinea pig on 20 gm., one rabbit on 40 gm., and one rabbit on 50 gm., failed to kill in the short time reported by Cunningham. Very stale meat in large and repeated doses failed to kill four animals. Decomposition does not seem to be responsible, nor is time or autolysis needed to develop the toxicity of the thyroid.

Considered as protein, the dosage of other materials used was enor-

mous for the body weights; the dosage of thyroid in most cases was much smaller. The dosage of desiccated material reached 2 gm. per day for most tissues and for all of the animals used at some time; rats received 2 gm. of kidney per day for twenty days, guinea pigs and rabbits received 2 gm. of liver, spleen, and brain for from twenty-one to fifty-six days. One guinea pig and two rabbits each received 20 gm., another rabbit received 50 gm., of stale meat repeatedly, all without effect. These dosages are from two to six times as great as the dosages of thyroid used and probably many times as great as the minimum fatal dose for these animals. The time of observation with negative results is four or five times that needed for fatal results with thyroid. The toxicity would seem to depend on something more than an excess of proteid.

CONCLUSIONS.

1. Thyroid in the forms used — fresh, stale, and desiccated, either commercial or laboratory prepared — contains a substance that is decidedly toxic for some animals.
2. The other animal tissues used — brain, liver, spleen, kidney, and skeletal muscle — give no evidence of toxicity when prepared and fed in the same way in equal or even larger quantities.
3. While the study does not indicate the nature of the toxic substance, it would seem to show conclusively that it is not due to autolysis or decomposition, and that it is not simply an excess of protein in the food.

FURTHER STUDIES OF THE ACETO-NITRILE TEST FOR THYROID SUBSTANCE IN THE BLOOD.

By HERBERT O. LUSSKY.

[From the Hull Physiological Laboratory of the University of Chicago.]

I. INTRODUCTORY.

THE chief aim of this investigation was to determine more specifically whether the aceto-nitrile test of Hunt is a specific test for thyroid secretion.

Much work on the physiology of the thyroid gland has recently been done in this laboratory by Professor Carlson and his pupils. The results of one series of experiments with thyroid feeding have just been recorded. If the Moebius theory of the relation of the thyroid secretion to the symptoms of exophthalmic goitre is correct, these symptoms ought to be produced in laboratory animals when placed in a condition of hyperthyroidism, if such a thing is possible. Accordingly large amounts of desiccated dog's and sheep's thyroids were fed to dogs, cats, foxes, monkeys, guinea pigs, rabbits, rats, pigeons, chickens, and ducks. It was found that dogs, cats, foxes, and ducks are very resistant, while rabbits, pigeons, rats, guinea pigs, monkeys, and man show a greater susceptibility. The toxic effect produced by feeding is within limits specific for the thyroid and is greater for normal than for hyperplastic thyroids. This latter finding is entirely in accord with our expectations, since the percentage of the supposed active principle (iodine compound) is greater in the normal than in the hypertrophied gland.

Large amounts of desiccated thyroid were fed to dogs, cats, and ducks for months without producing symptoms with the possible exception of slight emaciation. One can account for the lack of more specific symptoms in but three ways. Firstly, the material constituting the active secretion is not absorbed as such. This, however, cannot be the

case, as is shown by the effect of thyroid feeding in clinical and experimental hypothyroidism. Secondly, the lack of symptoms might be due to a lack of excessive accumulation of the secretion in the blood. The rate of absorption and the rate of elimination of the thyroid substance from the blood after absorption might be so related to each other that there is at no time a sufficient concentration of thyroid in the blood to produce symptoms. In other words, there may be a rapid process of absorption going on simultaneously with a rapid process of elimination, or a slow process of absorption with a slow or rapid process of elimination or destruction. It is evident, however, that although the concentration of thyroid never becomes great enough to produce symptoms it must at some time be greater than normal, and could be detected if we possessed sufficiently delicate methods. Thirdly, the thyroid secretion may be absorbed in its active form from the digestive tract, and an excessive thyroid feeding may reach an excessive concentration in the body fluids, but certain species may exhibit no symptoms from this excess.

It was upon the suggestion and under the direction of Professor Carlson that we undertook this work to determine if with our present tests an increase or decrease in the concentration of thyroid secretion can be detected in the blood of animals in which the amount of thyroid substance has been altered.

II. LITERATURE.

No one has as yet been able to demonstrate the supposed secretion of the thyroid in normal mammalian blood. Even Hunt's aceto-nitrile test, which at present appears to be the most delicate test for some substance in the thyroid, gives negative results.¹ Likewise, the lymph coming directly from the thyroid parathyroid gland complex, which according to the older views ought to contain a greater concentration of thyroid secretion than the blood, has been shown by Carlson and Woelfel² to give no evidence of a secretion.

On testing the blood of guinea pigs after prolonged and excessive thyroid administration, when we should expect increased quantities of thyroid or thyro-iodine in the blood, Hunt³ obtained negative re-

¹ HUNT and SEIDELL: Hygienic Laboratory, 1909, Bulletin No. 47.

² CARLSON and WOELFEL: this Journal, 1910, xxvi, p. 32.

sults. In the blood of two cases of exophthalmic goitre, however, Hunt³ reports finding evidence of the secretion, while in a third the results were negative. Carlson and Woelfel² also report negative results in the blood of one case of exophthalmic goitre.

The intravenous injections of thyroid preparations have so far produced no symptoms which can be traced specifically to thyroid. It is evident in the light of the present knowledge of the action of tissue extracts on the circulation that the lowering of blood pressure and the slowing and strengthening of the heart beat are not due to the specific action of thyroid upon the heart and blood vessels, as is assumed by Von Cyon and Oswald.⁴ It is more probable that traces of cholin in the preparation are responsible for these blood pressure changes.⁵

Olds⁶ found that rats show no increased resistance to morphine after thyroidectomy, contrary to what one would deduce from the fact that thyroid administration decreases the resistance of rats to this poison. Likewise, Hunt could find no increased resistance in guinea pigs to aceto-nitrile after thyroidectomy.

Trendelenburg⁷ found that the blood of thyroidectomized cats increases the resistance of mice to aceto-nitrile. He concludes that, in the absence of the thyroid, toxic metabolic products accumulate in the blood, and that these products are responsible for the increased resistance of mice to aceto-nitrile. These substances, when absorbed by the thyroid, cause the latter to give the reaction.

III. METHODS.

The work was divided into two sets of experiments: the one was concerned with producing a decrease of thyroid secretion in the blood; the other with producing an increase.

As a test for the secretion of the thyroid we used the aceto-nitrile test of Hunt, since by this means as small a quantity as 0.1 mgm. of

³ HUNT: *Journal of the American Medical Association*, 1907, xlix, p. 240.

⁴ VON CYON and OSWALD: *Archiv für die gesammte Physiologie*, 1901, lxxxiii, p. 199.

⁵ VON FÜRTH and SCHWARZ: *Ibid.*, 1908, cxxiv, pp. 113, 361.

⁶ OLDS: *this Journal*, 1910, xxvi, p. 354.

⁷ TRENDELENBURG: *Biochemische Zeitschrift*, 1910, xxix, p. 396.

thyroid can be detected. The test consists in feeding thyroid to white mice for a period of about eight days and then testing their resistance to aceto-nitrile. If certain quantities of thyroid are fed, the resistance of the mice is increased, in some instances as much as twenty times. We therefore expected to find an increase in the resistance of mice to aceto-nitrile after being fed on the blood of animals in experimental hyperthyroidism. Inasmuch as the Hunt test does not show the presence of the secretion of thyroid in normal blood, it cannot be hoped to show any difference between normal blood and the blood of completely thyroidectomized animals, although the thyroid secretion in the latter must necessarily be diminished. In the mouse itself, however, the normal immunity to this poison ought to be decreased by thyroidectomy.

For the first set of experiments gray house mice were used exclusively, since they withstand the operation of thyroidectomy better than the white mice and show the same increased resistance to aceto-nitrile on feeding thyroid. All of the gray mice were caught in the Biological Laboratories and kept in cages under identically the same conditions of care and feeding for a period of about one week. The full-grown healthy mice of about the same size were then selected. Part of them were placed separate as controls and the rest thyroidectomized. After a period of time ranging from three to eight weeks, during which all of the mice were kept under uniform conditions, their resistance to aceto-nitrile was tested.

In the second set of experiments we tested on mice the blood of dogs and rabbits after long and excessive administration of commercial sheep's thyroid by feeding; one sample of human blood after large doses of thyroid had been taken; the blood of dogs after intravenous injection of thyroid juice and intraperitoneal injection of thyroid emulsion; and the blood of thyroidectomized rabbits. For the intraperitoneal injections the ordinary commercial sheep's thyroid, after heating to 100° for several hours, was mixed with boiling 0.9 per cent NaCl solution. After cooling to body temperature it was injected. On the following day the blood for the tests was drawn. The thyroid juice was made by extirpating the thyroids (as nearly normal as possible) from dogs bled to death. The thyroids, as free from blood as they can possibly be obtained without perfusion, were then cut fine, ground with sand, mixed with Fuller's earth, and placed under 300 atmos-

pheres pressure. The liquid thus obtained was injected intravenously in dogs. Twenty-four hours after the injection samples of blood were drawn for the tests. Controls were run in this series by feeding thyroid juice to mice directly.

The mice used for these experiments were from closely related litters and had been kept under identically the same conditions of care and feeding since birth. The aceto-nitrile was dissolved in water and injected subcutaneously.

IV. RESULTS.

1. *Thyroidectomy does not decrease the resistance of gray mice to aceto-nitrile.* — Most of the gray mice survived the operation of thyroidectomy. The largest percentage of deaths seemed due to the anaesthesia during the operation. Very few showed respiratory symptoms due to injury of the recurrent laryngeal nerves and only two were seen in tetany. The only way of distinguishing between the normal and the thyroidectomized mice was by the scar. On testing their resistance to aceto-nitrile the results were remarkably uniform, in consideration of the fact that the mice could not possibly have been of the same age. Experiment I shows that there is no decrease in the resistance of gray mice to aceto-nitrile after thyroidectomy.

2. *Feeding thyroid to dogs does not increase the thyroid substance circulating in the blood to a detectable amount.* — A dog of about 8 kgm. was fed daily 20 gm. of desiccated sheep's thyroid mixed with ground meat from October 1 to December 1, 1910, when it was placed on a diet of 30 gm. per day until December 31. Two hundred and thirty-five cubic centimetres of blood were then drawn, desiccated and fed to mice mixed with cracker dust in the form of cakes; each mouse received half a gram of dried blood daily for eight days. The normal mice received cakes made of cracker dust only. The result is recorded as Experiment II. From December 21 to January 6, 1911, no thyroid was fed to the dog. But from January 6 to the 21st 40 gm. were fed daily. Two hundred cubic centimetres of blood were then drawn, desiccated and fed in cracker cakes to mice. The result is recorded in Experiment III. These two experiments show that the resistance of the mice to aceto-nitrile was not increased by feeding the dog's blood. This would simply mean that the amount of thyroid circulating in the blood of the dog which had received excessive amounts of thyroid for

such a long period of time could not be detected by this method. It does not prove that there was no increase in the concentration of thyroid in the blood over normal, since our test might not be sufficiently delicate to detect such a slight variation in amount as may be present.

3. The blood of thyroid-fed rabbits does not increase the resistance of mice to aceto-nitrile to an appreciable degree. — Five healthy rabbits were each fed 2 gm. of desiccated sheep's thyroid for eight days when they showed marked symptoms of emaciation, weakness, and diarrhea. Their blood was then drawn, desiccated and fed to mice in cracker dust cakes for eight days; each mouse received half a gram of desiccated blood per day. The result is recorded in Experiment IV. It was unfortunate that the number of mice was so limited that we could not obtain the lethal dose of the blood-fed mice more accurately. But it is evident that it must have been below 0.55 mgm. per gram weight of mouse. Thus the increase in the resistance of the blood-fed mice over the normal is so extremely small that it cannot be considered as an indication of thyroid in the blood which was fed.

4. The intraperitoneal injection of thyroid. — For these tests 30 gm. of commercial desiccated sheep's thyroids were heated at 100° for several hours and then mixed with boiling 0.9 per cent NaCl solution. After cooling to body temperature this emulsion was injected under aseptic conditions into the peritoneal cavity of a dog. A few hours later the animal showed signs of great depression and extreme weakness, and it remained in this condition until death. The following day samples of blood were drawn, desiccated, mixed with cracker dust in the form of cakes and fed to mice; each mouse received .75 gm. of desiccated blood daily for eight days. The resistance of the mice to aceto-nitrile was then tested. The controls were fed crackers in the form of cakes. The results, recorded as Experiment V, show that there is no marked increase in the resistance of the fed mice over the normal.

5. The intravenous injection of thyroid juice. — Seventy cubic centimetres of thyroid juice were slowly injected into the leg vein of an 8 kgm. dog. Immediately the dog began to show signs of marked depression; the blood pressure fell, and the heart beat became very slow. Both, however, returned to normal shortly after the injection. Twenty-four hours after the injection a sample of blood was drawn, desiccated and mixed with cracker dust and fed to mice for eight days, each mouse

TABLE I.

No. of exp.	No. of animals and food.	Maximum dose in mgm. per gm. wt. mouse tolerated.	Minimum fatal dose in mgm. per gm. wt. mouse.
I.	Seventeen thyroidectomized mice	0.60	0.54
	Ten normal mice	0.58	0.55
II.	Ten mice fed 4 gm. desiccated blood of dog which had daily received 20-30 gm. desiccated thyroid for eighty-three days	0.23	0.18
	Five normal mice	?	0.20
III.	Eleven mice fed 4 gm. desiccated blood of a dog which had daily received 40 gm. desiccated thyroid for fifteen days	0.35	0.29
	Nine normal mice	0.28	0.29
IV.	Seven mice fed 4 gm. desiccated blood of rabbit which had been fed desiccated thyroid until symptoms appeared	0.48	0.55
	Nine normal mice	0.33	0.30
V.	Five mice fed 6 gm. desiccated blood of a dog which had received 30 gm. of desiccated thyroid intraperitoneally	0.48	0.55
	Seven normal mice	0.33	0.30
VI.	Three mice fed 4 gm. desiccated blood of dog which had received 75 c.c. of thyroid juice intravenously	0.49	0.58
	Three mice fed 4 gm. desiccated blood of dog which had received 100 c.c. of thyroid juice intravenously	0.46	0.60
VII.	Five normal mice	0.48	0.39
	Four mice fed 2.1 gm. desiccated blood of dog which had received 50 c.c. of thyroid juice intravenously	0.40	0.45
VIII.	Eleven normal mice	0.30	0.25
	Three mice fed 8 c.c. of thyroid juice	0.70	?
IX.	Three mice fed 2.6 c.c. of thyroid juice	0.60	0.71
	Three mice fed 0.8 c.c. of thyroid juice	0.55	0.26
X.	Six normal mice	0.38	0.30
	Five mice fed 3.4 gm. desiccated blood of thyroidectomized rabbits	0.60	0.50
X.	Six mice fed 3.4 gm. desiccated blood of normal rabbits	0.47	0.50
	Six normal mice	0.25	0.30
X.	Five mice fed 3.8 gm. desiccated human blood which was drawn after eating 22 gm. of thyroid	0.90	0.90
	One mouse fed 7.5 gm. of same blood	0.90	---
X.	Three mice fed 3.8 gm. desiccated normal human blood	0.78	0.80
	Eight normal mice	0.75	0.72

receiving .5 gm. of desiccated blood daily. One week later 100 c.c. of thyroid juice were slowly injected into the same dog without producing noticeable symptoms. Twenty-four hours after the injection, samples of blood were drawn, desiccated and fed to mice in cracker cakes, each mouse receiving .5 gm. of desiccated blood daily for eight days. On examination of Experiment VI, it will be seen that the smallest dose on which the blood-fed mice died is not very much higher than the largest dose on which the normal mice survived. The difference, which would undoubtedly have been smaller had we possessed more mice, is not great enough to be considered a positive test.

The blood of a 7 kgm. dog drawn twenty-four hours after an intravenous injection of 50 c.c. of thyroid juice was also tested out on mice. Each mouse received .5 gm. of desiccated blood for eight days. Experiment VII shows that the blood likewise produced no increase in the resistance of the mice great enough to be considered a positive test.

The thyroid juice itself, however, gave marked results, as is shown in Experiment VIII. Three sets of mice with three in each set were fed for eight days on cracker cakes containing thyroid juice. Each mouse of the first set received 1 c.c. daily, those of the second set one third of a cubic centimetre, and those of the third set one tenth. The controls were fed cracker cakes. The first set showed at least twice the resistance of the normal mice, the second set about one and one-half, while the third set showed no increase. These results indicate that the resistance of the mice varied with the amount of thyroid juice fed.

6. *The blood of thyroidectomized rabbits.* — The blood of three healthy rabbits, three weeks after thyroidectomy, was drawn and desiccated and fed to mice in cracker cakes; each mouse received .4+ gm. per day for eight days. Another set of mice received the same amount of desiccated normal rabbits' blood for the same length of time. The control mice were fed merely crackers. Experiment IX shows that the blood of the thyroidectomized rabbits more than doubled the resistance of the mice to aceto-nitrile, while the normal rabbit's blood did not show such an increase. This result is in harmony with Trendelenburg's findings on the blood of thyroidectomized cats.

7. *Human blood after eating large amounts of thyroid.* — Professor Carlson, before and after eating large quantities of desiccated sheep's thyroid, had some of his own blood drawn. This was desiccated and

fed to mice. Five of the mice received per day .45+ gm. of the blood (desiccated) drawn after eating the thyroid for eight days; one mouse received .93- gm. per day. Three mice were fed .45+ gm. of normal desiccated blood per day for eight days. Eight mice were fed only cracker cakes. Experiment X shows that if there was any increase in the amount of thyroid in the blood it must have been very slight. It

TABLE II.

Date.	Grams thyroid taken.	Body weight in K.	Pulse.	General condition.
Oct. 15	0	76.7	80	Normal; 135 c.c. blood drawn.
Oct. 17	2.0	76.8	75	Normal.
Oct. 18	2.0	...	82	Normal.
Oct. 19	2.0	76.0	90	Headache, weakness, perspiration.
Oct. 20	2.0	75.2	95	Headache, insomnia, nervousness, weakness, perspiration.
Oct. 21, 10 A. M. . .	2.0	74.6	100	
Oct. 21, 10 P. M. . .	12.0	...	110	{ Condition same as on the 21st; 150 c.c. blood drawn.
Oct. 22, 10 A. M.	74.0	114	

was unfortunate that more mice which had been fed the large quantities of blood drawn after eating the thyroid were not available, since in this case the minimal fatal dose could not be obtained. If the increase from the lethal dose for the normal mice (between .72 and .75 mgm. per gram weight mouse) to the lethal dose for the mice-fed blood after eating the thyroid (.90 mgm.) is considered a positive indication for thyroid in the blood, then we must also consider as positive the increase from the lethal dose for the normal mice to the lethal dose for the mice-fed normal human blood (.78 to .80 mgm.). This would mean that the normal blood of some individuals gives a positive test by this method, and therefore that we have no control for the blood of exophthalmic goitre patients.

The effect of eating the thyroid in the experiment is briefly summed up by Professor Carlson in Table II.

There was no diarrhea or diminished appetite. Beginning October

19, there was more or less continuous feeling of distress in the abdominal region and occasional acute abdominal pains. During October 20-23 the symptoms were sufficiently severe to render the subject unfit for work.

CONCLUSIONS.

1. The fact that thyroidectomized mice show the same resistance to aceto-nitrile as normal mice leads us to either of two different conclusions. First, the Hunt test is not a specific test for thyroid substances, since the products formed by other tissues (to take the place of thyroid secretions) give the same test. Or, secondly, in the normal blood there is such an extremely small concentration of thyroid that the decrease in the concentration which must necessarily follow thyroidectomy cannot be detected by this method.

2. If the results recorded on feeding to mice the blood of thyroid-fed and thyroid-injected animals have been correctly observed, they would indicate that the thyroid substance, since it was not detected in such blood, is either (1) quickly taken out of the blood, as was shown by Hektoen and Carlson,⁸ and Pettit and Carlson⁹ to be the case with antigens, or (2) is quickly excreted, as is the case with many drugs. The rate of elimination of the thyroid substance from the blood is now under investigation.

3. The increase in the resistance of mice to aceto-nitrile due to feeding the blood of thyroidectomized rabbits agrees with Trendelenburg's findings on the blood of thyroidectomized cats.

4. In view of the facts that thyroidectomized mice show the same resistance to aceto-nitrile as do normal mice and that there are other substances in the blood of thyroidectomized animals which cause an increased resistance, it would appear that the Hunt test is not a specific test for thyroid.

5. Inasmuch as there are, at least under certain conditions, other substances than thyroid in the blood which increase the resistance of mice to aceto-nitrile, and since these substances may vary in different individuals, or at different times in the same individual, the Hunt test

⁸ HEKTOEN and CARLSON: *Journal of infectious diseases*, 1910, vii, p. 319.

⁹ PETTIT and CARLSON: *Ibid.*, 1912, x, p. 43.

on human exophthalmic goitre blood lacks sufficient control. In the case of positive results it is impossible to say which substances are present.

The author wishes to express his thanks to Dr. A. J. Carlson for the valuable suggestions and stimulating interest shown during the progress of this work.

THE PRESENCE OF PROTHROMBIN AND THROMBOPLASTIN IN THE BLOOD PLATELETS.

By STANHOPE BAYNE-JONES.

[From the Physiological Laboratory of the Johns Hopkins University.]

THE rapid disappearance of the platelets in shed blood during the process of clotting and their constant presence as a nucleus for various thrombi are phenomena that have led physiologists and pathologists to attribute to these elements an important rôle in the coagulation of the blood. But although attention has long been directed toward the platelets as furnishing one or more of the factors of coagulation, yet statements with regard to this function continue to be expressed with some degree of reserve. The coagulation factors that they have been supposed to contain are (1) *prothrombin*, the antecedent substance of thrombin, or "fibrin ferment," and (2) a substance which accelerates coagulation.

The view that the blood platelets contain the mother substance of so-called "fibrin ferment" was demonstrated experimentally by Morawitz,¹ in 1904, when his researches led him to conclude that the platelets contain prothrombin (or thrombogen) in large amounts. He found that aqueous extracts of the platelets, after the addition of calcium, caused a solution of fibrinogen to clot. No mention is made as to whether or not the platelet extract was effective without the addition of calcium. He argues also that thrombokinese must have been present in his platelet extract, since otherwise they could not have caused clotting of the fibrinogen with the aid of calcium alone. It is obvious that this argument is valid only if we accept Morawitz's theory of coagulation, namely, that calcium and kinase must combine to activate prothrombin to thrombin. This view has been called into question recently, and we can only accept the experiments made by Morawitz as demonstrating the origin of prothrombin from the plate-

¹ MORAWITZ: *Archiv für klinische Medizin*, 1904, lxxix, p. 215.

lets. As far as his experiments are concerned, it remains uncertain whether or not they also contain thromboplastic substances. It seemed very desirable, therefore, to investigate this latter point and also to corroborate, if possible, Morawitz's findings in regard to the origin of prothrombin from the blood platelets. At Dr. Howell's suggestion I have undertaken the study of these two points. The results given below show quite conclusively that platelets contain both prothrombin and thromboplastin (thromboplastic substance).

METHOD — ISOLATION OF PLATELETS.

The manner of showing the presence of prothrombin in the blood platelets consisted in causing a reaction between filtered extracts of these elements and solutions of pure fibrinogen, without and with the presence of calcium. The fibrinogen was prepared in all cases from thoroughly centrifugalized oxalated dog's plasma, according to Howell's² modification of the method of Hammarsten. The calcium used was in the form of calcium chloride in a 6 per cent solution. The isolation of the platelets was effected through a modification of Morawitz's method of fractional centrifugalization, as follows:

Two hundred cubic centimetres of blood were drawn from the femoral artery of a dog through an oiled glass cannula into an equal amount of the saline oxalate solution described by LeSourd and Pagniez³ as an efficient preservative of the blood platelets. The solution consisted of sodium oxalate, 4 gm. dissolved in 1 litre of a solution of sodium chloride, 0.9 per cent. The final mixture of blood with this solution contained 0.2 per cent sodium oxalate. This mixture was quickly centrifugalized at 1600 revolutions per minute for ten minutes. At the end of this time, a little more than half of the slightly rosy, opalescent, supernatant plasma was pipetted off, leaving the sedimented red blood corpuscles and leucocytes and a considerable proportion of plasma. Microscopical examination of the portion of the plasma drawn off revealed a great number of well-preserved platelets, together with a few leucocytes. To remove the latter, this portion of the plasma was again centrifugalized at 1600 revolutions per minute

² HOWELL: this Journal, 1910, xxvi, p. 453.

³ LESOURD and PAGNIEZ: Journal de physiologie et de pathologie générale 1907, ix, p. 582, and 1911, xiii, p. 56.

for ten minutes, which caused the remaining leucocytes and red blood corpuscles, and, unfortunately, a large number of the platelets to be sedimented on the bottom of the tube. The supernatant plasma was decanted into clean tubes, and again centrifugalized at 3000 revolutions per minute for a half-hour or longer, at the end of which time a grayish-white layer was found adherent to the bottoms of the tubes. Upon microscopical examination, this layer was seen to be composed entirely of platelets more or less agglutinated. The plasma was poured off, and in order to insure the freedom of the platelets from all traces of plasma; a large quantity of 0.2 per cent saline-oxalate solution was added, and the platelets were stirred up in this fluid. From this suspension they were recovered by centrifugalization at 3000 revolutions per minute for about fifteen minutes. The supernatant liquid was poured off, and extracts of the platelets were made both in distilled water and in 0.9 per cent solution of sodium chloride. The saline extract was found to be inactive, which accords with the findings of Morawitz and others, while the most active extract was obtained by allowing the platelets from 200 c.c. of blood to be acted on by 2 c.c. of water for one hour.

A microscopical control was employed throughout, using as a stain for the preparations, Wright and Kinnicut's aqueous solution of brilliant cresyl blue (1-300) as described by Duke.⁴ In none of the final preparations of platelets were any leucocytes observed.

With these preparations it was found that the blood platelets contain a substance which, in the presence of calcium, causes the clotting of a solution of pure fibrinogen. The addition of tissue extract was not essential. A typical experimental result is as follows:

Experiment XI. — 1. 1 c.c. fibrinogen + 5 drops of a solution of pure thrombin = clot in two minutes.

2. 0.5 c.c. fibrinogen + 0.5 c.c. water = no clot or precipitate in forty-eight hours.

3. 0.5 c.c. fibrinogen + 0.5 c.c. water + 1 drop CaCl_2 = no clot in forty-eight hours.

4. 0.5 c.c. fibrinogen + 0.5 c.c. aqueous extract platelets = no clot in forty-eight hours.

5. 0.5 c.c. fibrinogen + 0.5 c.c. aqueous extract platelets + 1 drop CaCl_2 = clot in one hour fifteen minutes.

⁴ DUKE: *Journal of experimental medicine*, 1911, xiv, p. 265.

Spontaneous clotting of platelet-emulsion. — In addition to prothrombin, the platelets have been considered to contain all the factors of coagulation. Thus Schittenhelm and Bodong⁵ give experiments to show that emulsions of platelets clot spontaneously. If this were so, it would vitiate the conclusions drawn as to the coagulative action of extracts of platelets upon fibrinogen. Special attention was given to this point, and it was found that in no case was there any sign of clotting in the extracts and emulsions of the washed platelets in either water or saline solutions. The result given by Schittenhelm and Bodong can be understood only on the assumption that their emulsion of platelets still contained sufficient plasma to cause clotting.

THROMBOPLASTIC ACTION OF PLATELETS.

The second object of these experiments was to investigate the accelerator action upon coagulation ascribed to the blood platelets. Morawitz considered that these elements contain a substance which, as in the case of extracts of other cells and tissues, hastens clotting. This substance he called *thrombokinase*, supposing it to act as a ferment activator after the manner of enterokinase. In his recent work on blood clotting, however, Howell⁶ has shown that this substance accelerates coagulation not by an action upon thrombin or prothrombin, but by neutralizing the antithrombin present, and he has proposed for it the name of *thromboplastin*. For the purpose of observing the thromboplastic action of the platelets, aqueous and saline extracts of them were added to a strong antithrombin plasma, obtained by injecting Witte's peptone (0.5 gm. per kilo of body weight) into the circulation of a dog. The blood, thus rendered incoagulable, was drawn after twenty minutes from the carotid artery through an oiled cannula, and was thoroughly centrifugalized. The preparations obtained in this way vary in their power of clotting, according as the peptone injection has caused the formation of much or little antithrombin. The best preparations do not clot at all — or at least the plasma obtained from the blood by centrifugalization, does not clot spontaneously, and even dilution with two or three times its volume of water may be

⁵ SCHITTENHELM and BODONG: *Archiv für experimentelle Pathologie und Pharmakologie*, 1905, liv, p. 223.

⁶ HOWELL: *this Journal*, 1911, xxix, p. 187.

ineffective. Tissue extracts will cause clotting in such plasmas, when added in sufficient amounts, and their action is quick or slow in proportion to the amount of antithrombin present. In this connection it is interesting to note that this peptone plasma can, by slow evaporation in watch crystals, be dried down and preserved in a desiccator for any length of time. The residue, taken up with an amount of 0.9 per cent salt solution equal to the original amount of the plasma, can be used in all respects like fresh "peptonized" plasma, and thus a supply of antithrombin plasma of constant strength can be kept on hand. When added to such peptone plasmas, extracts of platelets caused clotting. This result was obtained alike with aqueous and with saline extracts. Simultaneously, similar tests were made with extracts of washed red blood corpuscles, extracts of perfused turtle muscle and a special thromboplastin prepared from the glycerin extract of dried thymus, all of which caused clotting in the antithrombin plasma. To the thromboplastic action of all of these the platelets showed an equal strength. An experiment illustrating this fact follows:

Experiments Xa and XV. — 1. 1 c.c. peptone plasma + 1 c.c. extract thymus (undil.) = clot in four hours thirty minutes.

2. 1 c.c. peptone plasma + 1 c.c. H₂O = no clot in forty-eight hours.

3. 1 c.c. peptone plasma + 1 c.c. aqueous extract platelets = clot in one hour ten minutes.

4. 1 c.c. peptone plasma + 1 c.c. aqueous extract erythrocytes = clot in three hours.

5. 1 c.c. peptone plasma + 1 c.c. aqueous extract turtle muscle = clot in twenty-four hours.

This experiment shows that the thromboplastic action of the platelet extracts is more effective in neutralizing the antithrombin than extracts of the other cells. Other experiments, however, while all positive, did not emphasize this difference. Duplicate mixtures to those given above were made with the addition of a drop or two of calcium chloride (6 per cent). In these tubes clotting occurred only after a considerably longer time, or in some cases there was an entire failure to clot, owing to the inhibitory action of the calcium. Although these extracts were filtered before using, it might be objected that the coagulation was induced by the presence of particles acting as mechanical centres for the deposition of fibrin. This objection was eliminated by

the use of control tubes, in which suspensions of fine lint shreds and dust were added to some of the same peptone plasma without causing clotting.

SUMMARY.

1. By the use of solutions of pure fibrinogen and by blood platelets isolated in purity, it is shown, in corroboration of Morawitz, that these latter elements contain a substance (prothrombin) which, after activation with calcium, clots fibrinogen.

2. Extracts of the platelets also contain a substance (thromboplastin) which causes the clotting of so-called peptone plasmas, presumably by neutralizing the antithrombin present in such plasmas.

3. On the basis of these facts it may be concluded that the disintegration and solution of the platelets when blood is shed are helpful or essential to the clotting of blood in two ways: (1) by setting free a quantity of prothrombin which is subsequently activated to thrombin, and (2) by liberating a thromboplastic substance (thromboplastin) which neutralizes the antithrombin normally present in the blood.

SOME OBSERVATIONS ON DECEREBRATE FROGS WITH
ESPECIAL REFERENCE TO THE FORMATION OF ASSO-
CIATIONS.

By THEODORE C. BURNETT.

[From the Rudolph Spreckels Physiological Laboratory of the University of California.]

IT is generally assumed by physiologists and psychologists that the association processes are a function of the cerebral hemispheres. This is due to the fact that all the observations of workers in this field go to show that loss of the cerebral hemispheres is accompanied by loss of associative memory. Here and there, however, an independent thinker registers a doubt when such a statement is made. For instance, Loeb¹ says: "Associative processes occur everywhere in the hemispheres (and possibly in other parts of the brain)." So far as I have been able to ascertain, no work has been done on the positive side of this question; no one has made the attempt to prove that the lower centres may have the power of forming associations, when the higher centres in the cerebral hemispheres are removed. In a conversation with my friend and chief, Professor S. S. Maxwell, some time ago, he suggested that it might be worth while to try the method employed by Yerkes in demonstrating the presence of associations in normal frogs, and so prove definitely that a decerebrate frog could or could not form associations. This paper is the result of that suggestion.

At the outset considerable difficulty was encountered in getting the frogs to live after removal of their cerebral hemispheres. All the frogs we were able to get were from a source where the infection "redleg" was prevalent, and many of the deaths were due to that disease. The plan was finally adopted of isolating the most promising frogs and keeping each one in an aquarium jar by itself for three or four weeks before operating. By such means it was possible to eliminate this source of trouble. After operation, the frogs were kept in an environ-

¹ LOEB, J.: Comparative physiology of the brain, 1900, p. 275.

ment approaching as nearly to the natural as possible and still keep them in confinement. A large zinc-lined packing case, in which chemicals had been shipped from Germany, was divided into two compartments by a zinc partition, soldered securely and made water-tight. One compartment was filled with soil and then sown with grass seed. Slips of geranium and other plants were also set out in this miniature garden. The other compartment was filled with water, which was easily renewed when necessary, an outlet having been provided by which the water could be drawn off. The sides of the case were sufficiently high to prevent the frogs jumping out, but as an extra precaution a cover of coarse wire netting was provided. This made an ideal habitat for both the decerebrate frogs and the normal which were kept for comparison.

Three frogs were used in these experiments. One, a *Rana pipiens* of small size, was operated on May 1, 1911, and designated as D. Another (*Rana Boylii*), designated as D1, was operated on October 24, 1911, and the third (a larger *Rana pipiens*), D2, November 24, 1911. Observations on D began at the end of the summer vacation, and continued until his death, on January 4, 1912. D1 died January 2, 1912. Three normal frogs were selected as controls, as nearly like the decerebrate as possible, and were designated as N, N1, and N2 respectively.

Before proceeding to the main object of this communication, it may be well to point out certain differences that have been observed between the normal and decerebrate frogs, even at the risk of repeating some statements that have been made before.

In the first place, as to spontaneity. Any one casually observing a decerebrate frog would say there was no difference between such a frog and a normal one. But if a normal and a decerebrate frog are observed at the same time, it will be seen that there is a difference in degree. The decerebrate frog is somewhat less spontaneous. This was very well illustrated in the labyrinth to be described hereafter. When a normal and a decerebrate frog were put in the labyrinth together, the normal frog would at once start forward in his efforts to escape. The decerebrate frog, however, would remain quiet for varying lengths of time before it would start forward. This was rather a drawback to the experiments with the labyrinth, as it required constant stimulation to keep the decerebrate frogs moving. It was also noticed that the

decerebrate frogs spent a great deal more time in the water than did the normal. Possibly they were less subject to external stimuli under these conditions. On several occasions they were taken from the water and placed with the normal frogs. Sometimes they would jump back again without any apparent cause; at other times the slamming of a door or jarring of the building would be followed by a "plump" as the decerebrate frogs jumped into the water. If a decerebrate frog happened to be "sitting on the bank," so to speak, and one approached suddenly or made a sudden gesture, it would immediately spring into the water, especially if oriented in that direction. If oriented in some other direction, the frog might turn, but more likely it would spring forward, come in contact with the wall of its habitat, turn, and take to the water. The normal frog, on the contrary, was just as likely to crouch and remain perfectly motionless under these circumstances as to take to the water. One could not predict what would happen. It should be borne in mind that there are individual variations among decerebrate frogs as well as among the normal, and these statements are based upon observations upon a number of frogs extending over several months.

It was soon noticed that the decerebrate frogs were suffering from lack of nutrition. They were growing progressively thinner, while the normals did not seem to suffer particularly from this cause. Naturally the supposition was that the decerebrate animals were not getting the proper amount of food, and means were taken to insure this result. The decerebrate frogs were taken every day at noon and placed under a glass dish. Flies with one wing removed were introduced, and the frogs left there until all the flies had been caught. In addition to insuring their getting food, this gave an opportunity for comparison with the normal frogs as regards the feeding reflex. Incidentally it was thought that some insight into the matter of the formation of associations might be gained in this way, as A. G. Schaeffer² has recently shown that the feeding associations are readily learned. Schaeffer's interpretation of his results may have to be revised on the basis of inhibitions, but at present that does not concern us. Schaeffer's paper appeared too late in the season for hairy caterpillars, but on one occasion a belated one was found and put under a glass dish with decere-

² SCHAEFFER, ASA G.: *Journal of animal behavior*, 1911, i, p. 309.

brate frog D, at 12.15 P. M. At 12.18 D caught the caterpillar and promptly expelled it. At 12.20 D again snapped at the caterpillar, just touched it, and refused to take it. No more attention was paid to the caterpillar for ten minutes, and then at 12.30 D again snapped at it, caught and swallowed it. Three flies were introduced about ten minutes later, and in three minutes D had caught them all. A few days after another caterpillar was found, and this time it was offered to a normal frog under exactly similar conditions. In less than five minutes the frog had caught and swallowed the caterpillar at the first attempt. Further experiments along this line were not made; they are simply introduced here as being rather significant and worth more extended study. A description of a few experiments will suffice to make clear the difference observed between the decerebrate and the normal frogs as regards their feeding reflex.

September 18, 1911. — D and N were put under a glass dish with five flies. N crouched and paid no attention to the flies. D, on the contrary, caught three flies in rapid succession. N then caught a fly, and soon after D caught the fifth fly, making four caught by the decerebrate frog, while the normal frog caught but one. This was at noon. At 2.15 both frogs were again put under the dish with seven flies. D captured them all in about ten minutes. During the whole experiment N either crouched or made ceaseless efforts to escape, jumping at the sides of the dish or "boring" at the angle made by the edge of the dish with the table.

September 28, 1911. — 12.09 P. M. D and N with six flies and a grasshopper. At 12.10 D catches a fly. By 12.16 five flies have been captured by D. At 12.24 D captured the grasshopper. During all this time N has been making constant efforts to escape. The experiment was ended before the sixth fly was caught.

October 6, 1911. — 12.14 P. M. D and a different normal frog which had been under observation for some time and is unusually keen and active. Four grasshoppers. At 12.15 D caught a grasshopper. 12.17 D catches another. 12.18 and 12.22 N catches the remaining two grasshoppers.

October 7, 1911. — 12.10 P. M. D and the same normal frog as above, with six flies. D caught five flies as follows: one at 12.11, 12.13, 12.16, 12.24, 12.29. The normal frog paid no attention to the flies, but spent all the time in efforts to escape.

This will suffice to show the difference between the two frogs when in a strange environment. It is probable that fear inhibited to a certain

extent the feeding reflex in the normal frog, while the absence of fear in the decerebrate frog left the reflex unhampered. It was quite different in their natural environment. If flies with one wing removed were placed in the box where the frogs were kept, the normal frogs were even more alert than the decerebrate in capturing them, and more skilful and accurate. In spite of this care in feeding, however, the frogs did not gain, and, as stated before, died in January.

The decerebrate frog illustrates very well Loeb's³ theory of chain reflexes. Placed under a dish with a number of flies, D, for instance, would remain quite motionless until a fly crawled into his visual field. The retinal reflex at once oriented the frog toward the fly, and then followed one of two events. If the fly stopped and remained motionless, nothing further happened. If, however, the fly continued its movements, the orientation was immediately followed by the simultaneous spring and snap necessary to capture its prey, and then followed the swallowing. More than once, when D was not very hungry, he has been seen to sit in one spot and simply snap when a fly came into the visual field, without any attempt to capture it.

We come finally to the question, Can a decerebrate frog form associations? A few preliminary experiments made it very evident that the labyrinth used by Yerkes⁴ would not be practical. If a frog turned to the closed side, when stimulated, he would simply spring forward, or attempt to climb up the side of the labyrinth, until falling backward he would be oriented the other way. If still on the wires, the next stimulus would send him off, when he would, if unmolested, sit for a length of time proportional to the amount of stimulation he had undergone; for it is well known that decerebrate frogs fatigue quickly. It was determined therefore to modify the labyrinth in the direction of simplicity in the following way (see Fig. 1). The left side was closed entirely, so that the frog had no choice but to go forward toward the open end. The right side of the open end was closed by a glass plate. By this arrangement a single turn to the left was all that was necessary to escape. Additional wires were strung across the front end, so that if the frog remained too long at the glass his movements could be accelerated. It was also thought that this might form an association that was unpleasant. The region about the entrance to the labyrinth (X, Fig. 1) was also made uncomfortable by dropping ether on the frog from a

³ LOEB, JACQUES: *Comparative physiology of the brain*, 1900, p. 144.

pipette, if he lingered too long. There was only one really comfortable region, and that was in front of the open end, *O*.

In order to be sure the above method would work, it was first tried with a normal frog. As soon as he entered the labyrinth a drop of ether was let fall upon his back. The frog lost no time in moving on, but went in a straight line to the right side, which was closed with glass.

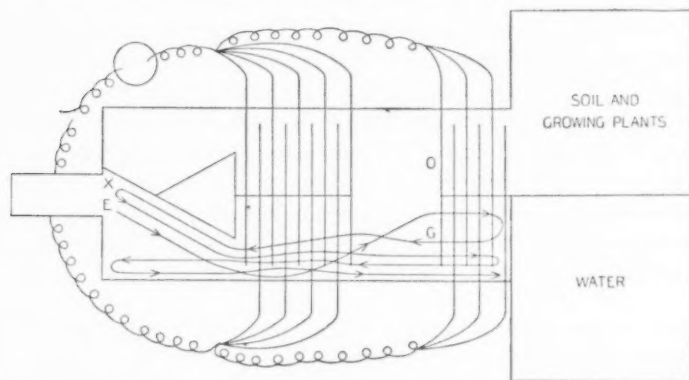


FIGURE 1. — A horizontal section of Yerkes' labyrinth as modified by the author. At *X* the left passage is closed, allowing no choice of direction. *E*, entrance; *O*, open end of labyrinth; *G*, end closed by a glass plate. *X* also marks the point at which ether was applied. The lines indicate the course of *D*₂ on the seventy-seventh trial.

Single induction shocks were then sent through the wires, whereupon the frog whirled about and jumped off to the clear space. After sitting quietly for a few minutes, he went to the open end and escaped. After about twenty trials this normal frog would make his escape with rarely an error. Occasionally he would make a mistake and go to the glass, but would correct the error on feeling the first shock. Undoubtedly he would have been perfect if the trials had been continued. In order to be sure this was an association, the labyrinth was reversed, the left opening being closed by a glass plate. This involved a turn to the right, instead of to the left as formerly. The frog went straight to the left side as usual, and finding his way blocked, tried to "bore" through the glass. An induction shock was followed by violent efforts to escape through the glass, until finally a sudden turn and jump sent him toward

⁴ YERKES, R. M.: Harvard psychological studies, 1903, i, p. 579.

the rear end. After sitting for a short time, the frog went to the rear end of the labyrinth, whence he started. It was quite obvious that he was at a loss how to escape.

The decerebrate frogs behaved in a very different manner. On entering the labyrinth they would jump forward when stimulated by the ether, and generally landed on the wires in the middle of the passage. If the induction shock was weak, they would either jump a short distance or crawl forward a short distance. If the current was strong, they would give a spring forward and land at the glass, or if oriented toward the open end, as often happened, they would spring to the opening and escape. If stimulated at the glass end, they would make frantic efforts to get through or over the glass, until, falling backward, they would be oriented toward the rear of the labyrinth. They would then go to the rear end, only to be made uncomfortable by ether, when back they would go again to the glass. The following notes on three different trials may be of interest.

January 2, 1912. — D2 was put in the labyrinth and went forward to the glass. Single induction shock sends him climbing up the glass. In doing so he loses his balance and falls backward, facing the rear. After sitting awhile, he turns and faces the opening. Stimulated with a drop of ether, he jumps forward and escapes. This was the first trial for this date, but the fifteenth of the series.

January 2, 1912. — Sixth trial for this date and the twentieth of the series. D2, forward to mid-wires. Stimulated, he jumps forward and turns to the left facing the opening. Turns to the right and goes to the glass. Stimulated, he climbs up and falls backward, facing the rear. The next jump carries him to the mid-wires, and then to the rear corner, whence he started. A drop of ether and he turns and goes to the glass again. Single induction shocks cause the same series of contractions by which this frog always responds, *viz.*, strong contraction of the extensors by which the frog is raised upright, with his belly against the glass, followed by loss of balance and falling backward facing the rear. By a slight turn to the right, the next jump brings him opposite the open end, but oriented away from it. After a few minutes, another turn to the right brings him to the wires at the open end. Here he sat for twenty minutes, when a single shock sent him off.

January 8, 1912. — Sixth trial for this date and the seventy-seventh of the series. On entering the labyrinth, a drop of ether sends D2 to the mid-wires. Stimulated, he goes to the glass. Stimulated, he reacts

as usual, falls back facing the rear, and then goes to the rear corner. A drop of ether again sends him straight to the glass without stopping. Stimulated, he again goes to the rear corner. A drop of ether sends him to the glass. By this time his toes were bleeding and he was quite fatigued; the experiment was therefore discontinued. The course of this frog is given in Fig. 1.

From the above it is quite evident that not a trace of association was exhibited by this frog. Over one hundred trials were made, but the last trial was no better than the first. What is true of D₂ is also true of the other two frogs, D and D₁. The death of the latter two cut short the experiments, but enough was done to make it reasonably certain that the same result would have been obtained.

From what has been stated herein, we may conclude —

1. That the reflex excitability of the decerebrate frog is heightened, owing to the loss of inhibitory influences from the higher centres.
2. From observations on the feeding reflex, Loeb's theory of "chain reflexes" receives additional proof.
3. The decerebrate frog is incapable of forming even the simplest associations, and Goltz's closing remarks still hold good: "The brainless frog is nothing more than a complex of simple reflex mechanisms."⁵

⁵ "Der enthirnte Frosch nichts ist als ein Complex von einfachen Reflex-Mechanismen."

THE BLOOD PRESSURE FALL PRODUCED BY TRACTION ON THE CAROTID ARTERY.¹

BY TORALD SOLLMANN AND EDGAR D. BROWN.

[From the Pharmacological Laboratories of Western Reserve University and of the
University of Minnesota.]

GENERAL PHENOMENA.

ONE of us (S.) noticed quite accidentally, in the course of a blood pressure experiment on a dog, that traction on the carotid artery in the neck caused a marked fall of blood pressure. This formed the starting-point of the present investigation. We found that the phenomenon occurs with uniform constancy in all the animals investigated (dogs, cats, and rabbits), unless there are interfering conditions, such as shock.

The fall as a rule begins almost simultaneously with the pull on the artery, or it may be delayed a few seconds, especially when the animal is in the condition of shock. The fall is somewhat abrupt, reaching the minimum blood pressure in ten to thirty seconds and returning to normal more gradually after traction is released. As a rule, the pressure reaches normal in thirty to one hundred seconds, depending upon the amount of fall (which in one instance (Tr. 130), was 100 mm. of mercury), and according to the general condition of the animal.

The heart rate in almost all instances where observations were taken was slowed by 3 to 25 beats per minute, even when the vagi were divided; in a very few instances it was quickened. There is at the same time a disturbance of respiration; in some instances it becomes slowed and more shallow (Tr. 108-141), whilst in others it is quickened and deeper. This disturbance is in a large measure governed by the degree of anæsthesia; respiratory phenomena being more likely to occur when the animal is lightly anæsthetized.

¹ A preliminary report on this subject was published by us in the Proceedings of the Society of Experimental Biology and Medicine, 1908, v, p. 20.

All the above-described phenomena could be obtained only by traction on the cephalic end of the common carotid artery, and not on the cardiac end. It was not materially affected by division of the vagi, cervical sympathetic and depressor nerves.

The site of the phenomenon was traced to the origin of the internal carotid arteries and the dense plexus of vessels and nerves in this region.

CONDITIONS GOVERNING THE PHENOMENON.

Our experiments were made in two series of animals: in Experiments 45 to 85 (phenol series) our usual method of procedure consisted in an-

TABLE I.
FALL OF BLOOD PRESSURE FROM CAROTID TRACTION.

Vagi .	Right side.	Left side.
Vagi intact	36	33.4
Vagi divided	25	26.2

æsthetizing the animals (morphine and ether for dogs and cats, urethane and ether for rabbits), exposing, tying, and dividing both carotids in the neck. The blood pressure was taken from the femoral artery in dogs, from the cardiac stump of the carotid in cats and rabbits.

In Experiments 1 to 32 (carotid series) dogs were anæsthetized with morphine and Grehant chloroform anæsthesia, cats and rabbits as in the phenol series. The blood pressure in this series was taken from the left carotid artery in all cases.

The figures given in the tables represent the fall in millimetres of mercury unless otherwise stated.

Comparison of the average fall in blood pressure on the two sides.—The average fall in blood pressure in thirty-three animals (thirty dogs and three cats), where traction was made on the two sides, shows they are practically equally effective (Table I).

Comparative effect of traction on one artery and on both at the same time.—From Table II it will be observed that the fall is somewhat greater

in most instances where traction is made on both arteries at the same time; but the difference is small, and the experiments are too few in number to make any further deduction.

TABLE II.

Dog.	Right artery.		Left artery.		Both arteries.	
	B. P.	Fall.	B. P.	Fall.	B. P.	Fall.
14	112+ ¹	30	110+	26	110+	44
..	120—	22	113—	20
55	205—	77	188—	70	180—	83
66	70—	11	68—	11	90—	22
..	190—	18	200—	38
..	130—	17	110—	20
73	58—	3	58—	4
Average	140.7	25.4	123	33.0
+ equals vagi intact. — equals vagi divided.						

Carotids open and ligated. — Traction on the artery might modify the blood supply of the brain, and this might be the cause of the fall.

This is readily excluded by the fact that the reaction occurs equally well or even better when the carotids have been ligated, as is shown in Table III.

Moreover, it is well known that mere clamping of the carotids produces a rise of blood pressure, and not a fall, a fact which is confirmed in our experiments.

The greater fall with ligated carotids may be due to the higher blood pressure.

The effect of blood pressure upon the amount of fall. — It was observed, while performing these experiments, that the fall was more pronounced when the blood pressure was high, which would naturally be expected; but the fall appeared to be relatively more diminished when

the blood pressure fell below 80 mm. Sollmann² has pointed out that this holds for a variety of blood pressure reactions. In order to determine the relation of traction fall to blood pressure, an average was taken from thirty-eight dogs (Table IV).

Hemorrhage. — In one animal (Dog 62) we tried the effect of traction after lowering the blood pressure by bleeding the animal, and then

TABLE III.

FALL OF PRESSURE FROM CAROTID TRACTION WITH CAROTIDS OPEN AND LIGATED.

Animal.	No.	Carotids open.	Carotids ligated.
Cat	56	40	40
Dog	62	70	50
Dog	63	12	35
Dog	19	29	80
Dog	20	12	20
Dog	21	20	60
Dog	22	28	60
Average		30.2	49.3

TABLE IV.

Blood pressure (mm.) . .	1-45	50-80	80-100	100-200	Above 200
Average fall	8.7	15	34.7	43.7	66.8

after raising the blood pressure again by returning the withdrawn defibrinated blood, or by the injection of normal salt solution. We found the traction fall could be repeatedly diminished or increased according to the height of blood pressure resulting from our treatment.

Opening the thorax. — When the thorax was opened, the response was generally diminished, the diminution corresponding approximately to the lowered level of blood pressure.

Table V shows the average results from ten dogs.

Degree of traction. — We also noticed that the blood pressure fall was somewhat modified by the amount of tension exerted upon the artery.

² SOLLMANN: *Journal of pharmacology and experimental therapeutics*, 1911, iii, p. 48.

In order to estimate the amount with some degree of accuracy, a string was either tied to the cephalic end of the severed artery, or, by means of a bent pin hooked around the undivided artery, run over a small pulley, and weights attached to the free end. Although different

TABLE V.

Before opening of thorax.		After opening of thorax.	
Blood pressure.	Fall.	Blood pressure.	Fall
140.4	34.7	97.6	27

animals may respond differently to carotid traction, Table VI indicates that about 200 gm. is the most suitable weight to give the maximum fall.

This weight was employed in a large number of animals, and gave practically the same degree of fall as that produced when traction was made by hand.

TABLE VI.

Weight (gm.)	20	50	100	200	400	500
Fall of blood pressure	0	23	38	72	..	53
	..	5	25	50	64	..
	90	..	67
	77	70	42
	13	22	5

Heart rate response to carotid traction. — The effect on the heart rate, as shown in Table VII, is interesting. Counting the average of the six experiments, carotid traction slows the heart, before section of the vagi, from 90.7 to 75.3 = 15.4 beats per minute.

After section of the vagi from 163.8 to 152.8 = 11 beats.

In three of the experiments (53, 67, 68) the numerical slowing is less after section of the vagi, but in two (63, 64) it is somewhat greater, whilst in one (62) it is practically unchanged.

The slowing therefore, is almost as great or quite as constant when

the vagi have been divided; so that stimulation of the vagus centre does not fully account for the slowing.

TABLE VII.

Animal.	Before section of vagi.	After section of vagi.
53	88-64 = 24	168-160 = 8
62	102-80 = 22	198-175 = 23
63	78-54 = 24	185-156 = 29
64	78-68 = 10	150-132 = 18
67	120-108 = 12	114-114 = 0
68	78-78 = 0	168-180 = +12

IS THE FALL OF BLOOD PRESSURE OF CARDIAC OR VASOMOTOR ORIGIN?

In order to show the relative share of the heart and vessels in the fall of blood pressure, we resorted to the myocardiograph, plethysmograph, and oncometer, to compression of the aorta, to division of the splanchnics, and to drugs.

Myocardiographic tracings (Fig. 1). — For these the anæsthetized animal was curarized and continuous oxygen insufflation (Hirsch) was

TABLE VIII.

Dog.	Blood pressure fall.	
64	25	Systole slightly but plainly diminished.
65	7	Systole slightly but plainly diminished, vagi intact.
65	7	Systole slightly but plainly diminished, vagi divided.
65	48	Systole markedly diminished, vagi divided.
67	17	Systole markedly diminished, vagi divided.

started; a rib was resected, the pericardium incised, and a pin hooked into the muscle of the ventricle. The cardiac movements were generally recorded by passing a string from the pin to a tambour which was connected with a second Brodie's tambour tracing on the drum (Table VIII). As a control, the pin was tied directly to a muscle lever in some of the tracings.

The myocardiograms show that the heart, especially the systole, is weakened during the fall; the fall and the weakening being synchronous. This does not decide absolutely which is cause and effect, whether the pressure falls because the heart is weakened, or *vice versa*.

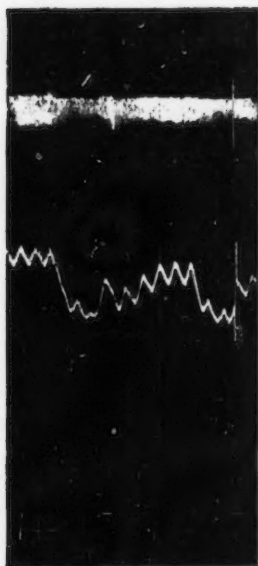


FIGURE 1. — Dog, 67; tracing, 170. Both vagi are divided. The upper tracing is a myocardiogram, written by a double tambour. The upstroke is diastolic. The lower tracing is the blood pressure. The base line shows zero pressure. The first signal corresponds to traction of both carotids, the second to traction of the right carotid.

Clamping the aorta. — In order to exclude as far as possible the possibility of part of the reaction being vasomotor, the aorta was clamped just below the left subclavian artery, and in two dogs the left subclavian was ligated. The average traction fall (12 experiments in three animals — Dogs 20, 21, 22) is practically the same with aorta clamped or open (Table IX).

This experiment shows quite conclusively that the vasomotor system takes no essential part in the phenomenon, for the traction fall is apparently unaltered when the greater portion of the vascular system is shut off.

Volume of organs as recorded by the oncometer and plethysmograph. — As a further means of determining whether the vasomotor mechanism was concerned in the phenomenon, readings were taken of the oncometric volume of the kidney and spleen, before and during the change in blood pressure.

These show a diminution in volume as the blood pressure falls.

TABLE IX.

Aorta open.		Aorta clamped.	
Blood pressure.	Fall.	Blood pressure.	Fall.
77.2	25.5	162.4	26.7

An oncometric tracing of the kidney (Fig. 2) shows the volume of the organ coincides almost identically with the blood pressure. Plethys-

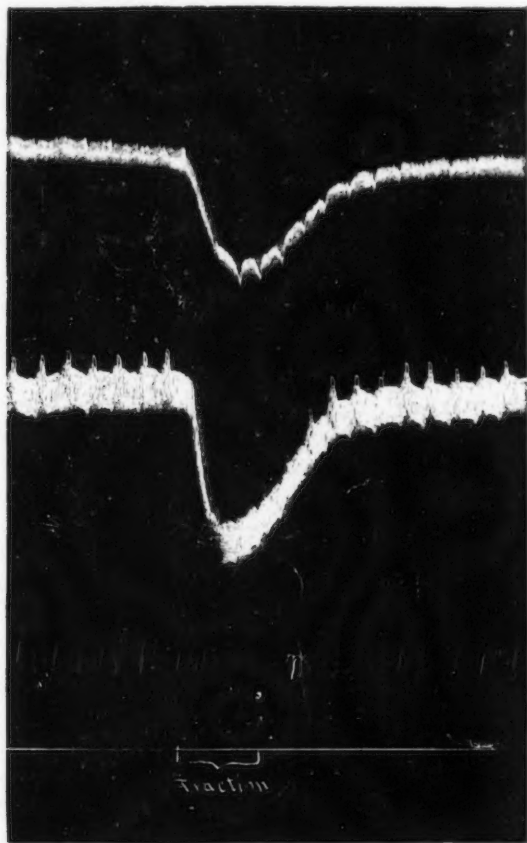


FIGURE 2. — The upper tracing is an oncometer curve from the kidney; the lower tracing is the blood pressure; the base line records zero pressure.

mograms of the front leg also showed the volume of the limb diminished with the blood pressure fall (Tracing 129). According to our present knowledge this would be considered conclusive evidence that the phenomenon is cardiac and not vasomotor.

Vasomotor centre (perfusion of innervated organs). — The reaction of the vasomotor centre was studied by the method described by Sollmann and Pilcher.³

The organ (generally the spleen) is perfused *in situ*, being entirely severed from the circulation of the animal, but the innervation being intact. Changes in the flow indicate corresponding changes in the central vasomotor tone.

This was tried on nine animals. Of these four showed a slight vasodilatation, whilst five gave no result.

The results of this method are therefore indecisive. This in itself makes it rather improbable that the fall is mainly vascular.

PRELIMINARY ATTEMPT AT LOCALIZATION.

Having ascertained that both sides are approximately equally effective (Table I) and that the vagus nerve is not directly involved, we endeavored to locate the origin of the reaction.

Since the phenomenon occurred primarily from manipulation of the artery itself, our first object was to determine whether the reaction originated within the arterial wall.

In order to settle this point we tried:

The relative efficiency of electric stimulation and of traction in the neck. — It was found that electric stimulation (Dogs 45 and 46) was practically ineffective even with very strong induction shock.

A very slight fall was observed, but not greater than could be produced by gentle manipulation of the artery, such as handling with the fingers or sponging of the wound (Tr. 151).

In these same animals traction caused a fall of 35 mm. and 55 mm. respectively.

Stretching the immobilized artery. — The artery in the neck was grasped in two places between the finger and thumb and the intervening portion stretched, without effect. The employment of hæmostats in like manner on various portions of the artery was ineffective.

It is therefore evident that the reaction does not originate within the arterial wall of this region. It being thus excluded that the phenomenon arises either from stimulation of the arterial wall or from cerebral anæmia, there only remains the possibility that the traction

³ SOLLMANN and PILCHER: this Journal, 1910, xxvi, p. 233.

is transmitted to some more distant structure, and there produces the effective stimulation.

This had to be located by successive stages, first, as to the direction in which it is transmitted.

Comparative efficiency of traction on the cardiac and cephalic ends of the artery. — It will be observed, by a mere glance at Table X, that the cephalic end is the one involved, and therefore the stimulation is transmitted solely in the cephalic direction.

TABLE X.
FALL OF BLOOD PRESSURE FROM CAROTID TRACTION.

Animal.	Cardiac end.	Cephalic end.
	mm.	
Dog 46	10	56
Dog 47	0	36
Dog 49	0	60

The next possibility was that the traction is transmitted to the vagus, since the nerve is so intimately associated with the artery. Were this the case, we should get even greater results from traction on this nerve. This, however, is readily excluded, for this procedure tended to produce a rise of blood pressure rather than a fall (provided that both vagi are divided).

Result of traction in the vagi (divided). — It will be seen, by Table XI, that the fall does not occur on traction of the vagus in the neck, which gives a slight rise, if anything, from the cephalic end, and a very slight and variable effect from the cardiac end.

Comparison of the fall in blood pressure before and after division of the vagi. — In order to determine whether there was really any material difference in the fall before and after section of the vagi, as is shown in Table I, especial attention was paid to this subject in a number of animals in our later experiments.

An average taken from nineteen animals shows that the fall is somewhat greater where the vagi were intact (Table XII), and the blood pressure tracings show indications of vagus stimulation (Tr. 19), which accounts for the great fall.

TABLE XI.

RESPONSE OF BLOOD PRESSURE TO TRACTION OF DIVIDED VAGI.

Animal.	Left cephalic.	Right cephalic.	Left cardiac.	Right cardiac.
Dog 49.	10 rise	15 rise	0	8 rise
Cat 56	23 "
Cat 56	8 "
Rabbit (vagi intact)	10 fall	20 fall

Another point in connection with section of the vagi which was observed in a number of animals was a complete loss of reaction to traction for a brief period after the section, the longest time noted (Dog 19) being fifteen minutes; this case, however, being unusually long. The only explanation that we are prepared to offer for this is that it is probably due to shock of the nervous mechanism with which we are dealing.

TABLE XII.

Vagi intact . . . 36.9

Vagi divided . . . 28.8

Traction on the depressor and cervical sympathetic nerves. — Traction exerted on the cephalic end of these nerves gave very little or no fall of blood pressure (0 to 10 mm., in Rabbits 60 and 61), so that they may be excluded as afferent paths.

Effect of division of the depressor and cervical sympathetic nerves. — Table XIII shows that the fall on carotid traction was at least as great when the nerves had been divided.

TABLE XIII.

RABBIT 60 (both vagi divided). After division of	Traction fall.
left sympathetic	90- 68 = 22
Both sympathetics and right depressor	130-100 = 30
Both sympathetics and both depressors	125- 60 = 65
RABBIT 61.	
Vagi intact, depressors intact	56- 52 = 4
Vagi intact, depressors divided	70- 64 = 6
Vagi intact, depressors divided	85- 50 = 35

Effect of stimulation and traction on the superior laryngeal nerves. — We tried the effect of electric stimulation and of traction on the central and

peripheral ends of the superior laryngeal nerve after dividing the vagi in order to determine whether these nerves were concerned in the reaction. This was tried on four dogs (26, 29, 30, 31) with quite uniform results. When traction was made on the central end, there was a rise in blood pressure from 4 to 7 mm. in two of the animals, whilst the

TABLE XIV.

SECTION OF ACCELERATOR NERVES.

	Before division.	After division.
Blood pressure fall	200-160 = 40	170-75 = 95
Rate slowed	192-150 = 42	132-120 = 12

other two showed no effect. Electric stimulation of the central end produced a rise in all animals from 7 to 100 mm. Electric stimulation and traction on the peripheral end was ineffective in all cases except Dog 26, where there was a fall in blood pressure from 8 to 15 mm. This excludes the possibility of the superior laryngeal nerve being concerned in the phenomenon.

It may therefore be concluded that the cervical nerves (depressor, sympathetic, vagus, and superior laryngeal) are not connected with the reflex, either as afferent or efferent paths.

Effect of division of accelerator nerves on the phenomenon. — In Dog 63 the annulus of Vieussens was divided on both sides. The results are shown in Table XIV.

Excision of both inferior cervical ganglia and of all branches. — The fall of blood pressure occurs fully after section of both the accelerators

TABLE XV.

EXCISION OF INFERIOR CERVICAL GANGLIA.

	Before excision	After excision.
Dog 73. Blood pressure fall	32	30

(Dog 63) and even after excision of both inferior cervical ganglia with all their branches (Table XV). This suffices to show that the efferent path is not through the annulus. This seems, however, concerned in the slowing of the heart, but it would be unsafe to draw this conclusion from a single experiment.

Effect of paralyzing and of removing the stellate ganglia. — In Dogs 1 to 4 the stellate ganglia were removed on both sides without puncturing the pleura, by an incision back of the shoulder over the junc-

tion of the second rib with the vertebra, a portion of the rib being removed.

Table XVI shows that the efferent path is not through the stellate ganglia, at least not in the main. The natural diminution of reaction

TABLE XVI.

REMOVAL OF STELLATE GANGLIA.

	Result.
Dog 1a, 1 per cent nicotine applied to ganglia	Reaction still present.
Partial extirpation of ganglia	Reaction markedly diminished.
Dog 3a, 1 per cent nicotine injected into ganglia	Reaction not abolished.
Incomplete extirpation of ganglia	Reaction much diminished.
Dog 4a, Ganglia completely extirpated	Reaction scarcely diminished.
Dog 2, 1 per cent cocaine injected into ganglia	Reaction scarcely diminished.
Dog 9, Stellate ganglia completely extirpated	Reaction slightly diminished.

with a lowered blood pressure makes it difficult to decide whether any part takes this path, but the result obtained in Dog 4a renders this improbable.

Division of the splanchnics. — The fall of blood pressure occurs almost as well after both splanchnics have been divided. The slight difference corresponds to the lowered blood pressure (Table XVII).

Several experiments were made, of which Dog 70 was the most successful. In this animal both splanchnics were divided just above the diaphragm.

TABLE XVII

DIVISION OF SPLANCHNIC NERVES.

Dog.	Intact.		One divided.		Both divided.	
	Blood pressure.	Fall.	Blood pressure.	Fall.	Blood pressure.	Fall.
67	122	18	110	20
69	110	40	74	17	52	12
70	85	12	90	12	75	8

It is evident that the splanchnics are not concerned in the fall of blood pressure.

Modification produced by depressor stimulation. — To determine whether the carotid traction fall has anything in common with depressor

stimulation, the two measures were combined. It seemed probable that if a maximal fall were obtained by one of these methods, the other should not further lower the blood pressure if it involved the same mechanism. If the mechanism of the two stimulations were different, it would be expected that the response would be additive.

This was the actual result. Thus in Rabbit 1 carotid traction alone lowered the blood pressure, on the average, by 17.5 mm.

During maximal depressor stimulation the traction further lowered the pressure, on the average by 11 mm., the difference being explainable by the lowered blood pressure.

Conversely, depressor stimulation alone lowered the blood pressure by 20 mm. (average). During maximal carotid traction the depressor stimulation further lowered the pressure by 14.5 mm.

It may therefore be concluded that the carotid traction reflex is absolutely distinct from the depressor reflex (Table XVIII).

The localization of the afferent path in the carotid plexus.— We showed, on page 97, that the reflex originates in the cephalic direction from the thyroid region of the neck, and that it does not involve the cervical nerves of this region.

We therefore traced the phenomenon up the course of the carotid artery, and of its branches, testing the response to traction and electrical stimulation of the vessels and their accompanying nerves at various levels. Four such experiments were made in dogs (52, 53, 54, 55) and two on rabbits (60, 61) with identical results. It would be useless to enter into the full details of the experiments. In all the animals the cervical nerves and carotids were divided in the neck, and the carotid exposed at the level of the origin of the internal carotid in dogs by an incision near the angle of the jaw; in rabbits by prolonging the median incision. By dividing successively the branches of the carotid artery, it was found that traction on the cut branches, with the exception of the internal carotid, was entirely or practically negative (Tr. 129, 130, 133); whilst traction on the internal carotid or on the stump of the common carotid, so long as it remained connected with the internal carotid, produced the typical phenomena on blood pressure and respiration. It is evident, therefore, that the traction phenomena are transmitted practically exclusively through the internal carotid artery. It was also found that electrical stimulation of the region about the internal carotid just outside the skull in one instance (Tr. 134)

caused a rise, and in another (Tr. 142) there was the typical fall, showing that the structures which are stimulated by traction of the carotid are situated in this region and not within the skull.

TABLE XVIII.
COMBINATION OF CAROTID TRACTION AND DEPRESSOR STIMULATION.

	B. P.	Vagi.	Traction fall.	Stimulation fall.	Further fall by traction.	Further fall by stimulation.
Rabbit 1.	82	Intact.	14
	74	Intact.	..	20
	88	Intact.	19
	94	Intact.	..	28	12	..
	96	Intact.	25	24
	109	Divided.	15
	80	Divided.	25
	66	Divided.	..	14	12	..
	70	Divided.	10	10
	80	Divided.	25	11
	64	Divided.	..	18	10	..
	69	Divided.	..	32	8	..
	70	Divided.	20	18
	68	Divided.	..	30	10	..
Rabbit 61.	58	Divided.	24	0
	55	Divided.	..	18	10	..

The internal carotid artery, from its origin to its entrance into the skull, is surrounded by a rich plexus of nerve fibres, running in a bundle of connective tissue, and apparently also partly within the walls of the artery. It appeared highly probable that these nerve fibres should constitute the afferent path; but in attempting to show this directly, we encountered an unexpected difficulty. We found, namely, that dissection or manipulation of this region, and especially division of tissue, would destroy the reaction entirely. Not infrequently we found

that traction or stimulation of an isolated bundle of tissue, or of the internal carotid artery, would produce a fair effect, but when, in order to trace the direction of the impulse, we placed double ligatures on the bundle and divided, we found that no reaction followed, whether we stimulated or pulled on the central or peripheral stump or on both together.

In one instance a portion of the artery 2 cm. long was isolated from all connections except the nerve plexus. Traction on this piece of artery in any direction and even stretching from both ends gave the typical fall, but when a ligature was tied around the nerve plexus traction became ineffective.

This can only mean that the division or the tying of a ligature around the nerve plexus destroyed the irritability of the path, *i. e.*, that it induced a condition of circumscribed shock. In support of this view a very slow recovery of the irritability was observed in some cases (Dog 55).

This inhibition, to which we shall recur presently, does not seem to set in equally readily in all animals. In some it is induced by the very first manipulation of the sensitive region, whilst in others a fairly complete and prolonged dissection can be carried out before the reaction disappears. On such animals we could ascertain that the phenomenon could be produced by stimulation or traction of the tissues (nerve plexus), even when separated from the internal carotid artery (Tr. 142), whilst stimulation or traction of the isolated artery might or might not be effective presumably according to the success of the isolation. This proves the assumption that the artery acts merely as a means for the mechanical transmission of the traction stimulus to surrounding nerve fibres. It was found, further, that successive strands of tissue each gave the reaction; in other words, that the phenomenon does not reside in any one of the nerves, but in a considerable number of the nerves composing the plexus.

In view of this fact, further attempts to isolate the afferent fibres from the complex network appeared fruitless, and we are unable to state at this time either the peripheral or the central connections of this afferent path.

The inhibition of the reaction by circumscribed shock, upon which we have touched, is not the least interesting of the phenomena exhibited by this reflex. From the fact that recovery from this shock may occur,

even when the inhibition of the reaction was complete, it follows that the majority of the efferent fibres cannot have been severed by the injury, but that they were completely inhibited by injury to other fibres of the plexus.

This indicates that the entire centre is inhibited, unilaterally, by injury confined to a fraction of the fibres going to it. On the other hand, the central inhibition has no tendency to spread to the other side, or to the vasomotor or respiratory centres, for the blood pressure and respiration are unaltered, and traction on the opposite carotid gives an undiminished response. The inhibition, as has been said, may either be sudden or gradual, early or late, and may differ in this respect even in the same animal.

During the performing of these experiments we resorted to the use of drugs, amongst them some whose action is well established; hoping that these might aid in localizing the nervous structures involved.

The results were not of so much value as we had hoped for; in fact, in some instances they render the problem more complex.

The drugs employed for this purpose were atropine, nicotine, curare, epinephrin, pituitary extract, and extracts of the thyroid and thymus glands. The results of these experiments will be given in a future communication.

SUMMARY.

1. Traction on the cephalic end of the carotid artery causes a fall in blood pressure.
2. The amount of fall is in a large measure governed by the height of blood pressure, the degree of traction exerted, and the general condition of the animal.
3. The vagus nervous mechanism is not to any great extent involved in the phenomenon, although it appears to exert some slight influence.
4. The fall is purely of cardiac origin as far as we are able to determine by the present methods of investigation.
5. The efferent path is not through any of the known cardiac nerves.
6. The afferent path is through the carotid plexus, and the impulse is transmitted to this structure through the intimate connection with the internal carotid artery.

RELATION OF CALCIUM TO THE CARDIO-INHIBITORY FUNCTION OF THE VAGUS.

By H. H. HAGAN AND J. K. ORMOND.

[From the Physiological Laboratory of the Johns Hopkins University.]

AT the suggestion of Dr. Hooker we undertook the following experiments to investigate the relation of calcium and potassium salts to the cardio-inhibitory function of the vagus. The experiments were made in the winter and spring trimesters of 1911, and the animals used in the first series were terrapins, while large turtles and frogs were used in the last series.

It has been pointed out by Howell¹ and also by Busquet and Pachon² that irrigation of the heart with normal saline solution (NaCl 0.7 per cent) causes the cardio-inhibitory function of the vagus to be suppressed, but that if the exposure to the saline solution has not been for too long a period irrigation with Ringer's solution will revive the vagus control. The latter workers stated that in their experiments the addition of very small amounts of calcium to the saline solution prevented the loss of the cardio-inhibitory function of the vagus. More recently Mines³ has again investigated the importance of calcium for vagus function with similar results. Our first series of experiments consisted of a repetition of this work.

TECHNIQUE.

The heart was isolated *in situ* without injury to the pericardium. Since, in most cases, the inhibitory fibres were found in the right vagus, the inflow cannula was inserted into a vein on the left side of the heart

¹ HOWELL: this Journal, 1906, xv, p. 280.

² BUSQUET and PACHON: Journal de physiologie et de pathologie générale, 1909, xi, pp. 807, 851.

³ MINES: Journal of physiology, 1911, xlii, p. 251.

and the outflow cannula was inserted into a large artery on the same side. All other vessels were ligated at a sufficient distance from the heart to prevent injury to the pericardium and vagus fibres. The inflow cannula was connected with a pressure apparatus, and the solution entered the heart at a constant and approximately normal pressure. The vagus was exposed by opening the carotid sheath and was stimulated by an electrical current from an induction coil. The calcium solutions used were made from a stock solution of crystals of calcium chloride in which the amount of calcium was determined by titration.

The following are the results of experiments on the terrapin when attention was confined to auricles and ventricles, since it was not possible to observe the sinus beat in the preparation here used. The numbers indicating the current strength are purely relative, as they represent distance between the primary and the secondary coils; the larger number indicates, of course, the weaker current. Inhibition in the table, unless the term is otherwise modified, means complete inhibition, that is to say, a complete cessation of the beat of the auricles and ventricles.

Experiment 1. —

Solution.	Current strength.	Inhibition.
Ringer's.	18	Yes.
NaCl 0.7%	16	No.
NaCl 0.7% CaCl ₂ 0.005%	16	No.
Ringer's.	18	Yes.
Ringer's (continued)	23	Yes.
NaCl 0.7%	1	No.
NaCl 0.7% CaCl ₂ 0.005%	1	No.
NaCl 0.7% CaCl ₂ 0.05%	22	Yes.
Ringer's.	23	Yes.
NaCl 0.7%	1	No.
NaCl 0.7% CaCl ₂ 0.05%	18	Yes.
Ringer's.	23	Yes.
NaCl 0.7%	1	No.
NaCl 0.7% KCl 0.03%	1	No.

Experiment 2. —

Ringer's.	22	Yes.
NaCl 0.7%	1	No.
NaCl 0.7% CaCl ₂ 0.025%	23	Yes.
Ringer's.	25	Yes.

Relation of Calcium to Cardio-Inhibitory Function of Vagus. 107

Solution.	Current strength.	Inhibition.
NaCl 0.7%	1	No.
NaCl 0.7% CaCl ₂ 0.0125	23	Yes.
Ringer's	23	Yes.
NaCl 0.7%	1	No.
NaCl 0.7% CaCl ₂ 0.00625%	1	No.
Ringer's.	30	Yes.
NaCl 0.7%	15	No.
NaCl 0.7% CaCl ₂ 0.00625%	15	Partial.
Ringer's.	28	Yes.
NaCl 0.7%	10	No.
NaCl 0.7% CaCl ₂ 0.01%	29	Yes.
Ringer's	11	Yes.

Experiment 3. —

Ringer's.	22	Yes.
NaCl 0.7%	15	No.
NaCl 0.7% KCl 0.06%	15	No.
Ringer's.	21	Yes.
NaCl 0.7% KCl 0.06%	15	No.
Ringer's.	21	Yes.
NaCl 0.7% KCl 0.12%	15	No.
Ringer's.	21	Yes.

Experiment 4. —

Ringer's.	22	Yes.
NaCl 0.7%	15	No.
NaCl 0.7% CaCl ₂ 0.00625%	15	Partial.
Ringer's.	26	Yes.
NaCl 0.7%	15	No.
NaCl 0.7% KCl 0.015% CaCl ₂ 0.00625%	28	Yes.
Ringer's.	28	Yes.
NaCl 0.7%	15	No.
NaCl 0.7% CaCl ₂ 0.00625%	15	No.
Ringer's.	27	Yes.
NaCl 0.7%	15	No.
NaCl 0.7% KCl 0.015% CaCl ₂ 0.00625%	28	Yes.
Ringer's.	28	Yes.
NaCl 0.7%	15	No.
NaCl 0.7% KCl 0.015% CaCl ₂ 0.003125%	25	Yes.
Ringer's.	26	Yes.
NaCl 0.7%	15	No.
NaCl 0.7% CaCl ₂ 0.003125%	15	No.

Solution.	Current strength.	Inhibition.
Ringer's.	24	Yes.
NaCl 0.7%	15	No.
NaCl 0.7% KCl 0.0075% CaCl ₂ 0.003125%	20	Partial.
Ringer's.	23	Yes.

Experiment 5. —

In this and the two following experiments the beat of the heart was each time restored to normal by irrigation with Ringer's solution and inhibited with a current strength of 21-25 before the effects of the special solutions were investigated.

Solution.	Current strength.	Inhibition.
NaCl 0.7% CaCl ₂ 0.003125%	15	No.
NaCl 0.7% KCl 0.0075%	15	No.
NaCl 0.7% KCl 0.0075% CaCl ₂ 0.003125%	15	No.
NaCl 0.7% KCl 0.015% CaCl ₂ 0.003125%	15	Partial.
NaCl 0.7% KCl 0.0075% CaCl ₂ 0.00625%	25	Yes.
NaCl 0.7% CaCl ₂ 0.00625%	25	Yes.
NaCl 0.7% CaCl ₂ 0.004166%	15	Yes.
NaCl 0.7% KCl 0.03% CaCl ₂ 0.003125%	15	Partial.

Experiment 6. —

Ringer's.	31	Yes.
NaCl 0.7% CaCl ₂ 0.004166%	15	No.
NaCl 0.7% KCl 0.03% CaCl ₂ 0.004166%	30	Yes.
NaCl 0.7% CaCl ₂ 0.004166%	15	No.

Experiment 7. —

Ringer's.	21	Yes.
NaCl 0.7%	15	No.
NaCl 0.7% CaCl ₂ 0.004166%	15	Yes.
NaCl 0.7% CaCl ₂ 0.003125%	15	No.
NaCl 0.7% KCl 0.03% CaCl ₂ 0.003125%	22	Yes.
NaCl 0.7% CaCl ₂ 0.003125%	15	No.
NaCl 0.7% KCl 0.03% CaCl ₂ 0.003125%	20	Yes.
NaCl 0.7% CaCl ₂ 0.003125%	15	No.
NaCl 0.7% KCl 0.03% CaCl ₂ 0.003125%	19	Yes.
NaCl 0.7% KCl 0.03%	15	No.
NaCl 0.7% KCl 0.12%	15	No.
Ringer's.	23	Yes.

CONCLUSIONS FROM THE ABOVE OBSERVATIONS.

1. Irrigation of the heart with normal saline (0.7 per cent NaCl) solution removes the cardio-inhibitory function of the vagus.

2. The vagus control of the heart can be restored by irrigating with normal saline to which is added calcium chloride in small amounts. The minimal amount or threshold value of the calcium necessary for this restoration varied somewhat in different animals, but in general (for the terrapin) it lies between 0.00312 and 0.00625 per cent.

3. A sodium calcium solution too weak in calcium to restore the vagus action will do so in the presence of a small amount of potassium chloride. In other words, the presence of potassium lowers the threshold value of the calcium necessary for vagus action.

4. A sodium potassium solution will not restore the vagus control of the heart.

At the conclusion of the above series of experiments it was suggested that there was a possibility of an error in our results in that no observations of the sinus venosus had been made. For it seemed possible that perfusing with a sodium chloride solution might cause the auricles and ventricles to take on a rhythm independent of the sinus, and that complete vagus inhibition of the sinus might result without affecting the other chambers of the heart. Furthermore it seemed possible that the addition of calcium to the saline solution might bring about a restoration of the normal sequence of the beat of the various chambers, and by so doing also restore the inhibitory function of the vagus for the entire heart. To test this idea we carried out another series of experiments on the terrapin, turtle, and lastly on the frog. In these our technique was slightly modified from that described for the first series, in that it was necessary to open the pericardium in order to observe the beats of the sinus. Five experiments were made upon terrapins such as had been used in the first series and three were made upon a fresh-water turtle (snapper). In these experiments the sinus beat was observed as well as that of the auricles and ventricle. In some of these experiments an attempt was made to record the contractions of the three chambers, but the result was not so satisfactory as direct observation, since the feeble sinus record was confused by the tug resulting from the ventricular contractions. As regards the point in question, these experiments were conflicting in their results. When the

terrapi's heart was perfused with the solution of sodium chloride, the rhythm of the heart beat was dissociated in various ways. In some cases the auricles and ventricle beat independently of the sinus, or the sinus and auricles followed in sequence, while the ventricle beat independently, or perhaps the sinus ceased to show visible contractions. When the vagus was stimulated under these conditions, it had a variable effect. In some cases the sinus was inhibited completely, while the auricles and ventricles continued to beat without change in rhythm or at a slightly slower rate. In other cases the sinus as well as the auricles and ventricles were unaffected by the vagus stimulation. In all cases, however, when the solution of sodium chloride was replaced by Ringer's solution, the regular rhythm of the heart beat returned, and with it the complete inhibition of all chambers upon vagus stimulation. It was not possible from this series of experiments to draw any satisfactory conclusions. It would seem that some unknown condition in the experiments was not properly controlled. Since the experiments of Busquet and Pachon were made upon frogs, it was determined to repeat their observations on these animals with the additional precaution of observing the beat of the sinus venosus. This series of observations gave decisive results. In the first experiment the combined vago-sympathetic trunk was stimulated. The heart was first perfused with a Ringer's solution, and the threshold value was determined for the current necessary to give complete inhibition of all chambers. The Ringer's solution was then replaced by a solution of sodium chloride (0.7 per cent), and it was found that stimulation of the vagus was entirely without effect upon the heart. When the Ringer's solution was again substituted, the vagus control of the heart promptly returned to its normal condition. In a similar experiment upon another frog a solution consisting of sodium chloride 0.7 per cent, and calcium chloride 0.0115 per cent, was perfused through the heart after the irrigation with sodium chloride. With this solution also the vagus effect which had disappeared was completely restored. In two other experiments upon frogs the intracranial portion of the vagus was stimulated alone in order to meet the possible criticism that the beat of the sinus when the vagus was stimulated during the perfusion with sodium chloride was due to the simultaneous stimulation of the accelerator nerve fibres. The following brief notes of the experiments are reproduced:

Relation of Calcium to Cardio-Inhibitory Function of Vagus. III

Experiment 18. — Large frog used. In this frog the skull was partly removed and the central end of the vagus dissected out. The inhibitory fibres were exposed at their exit from the brain, and the sympathetic fibres were cut below the vago-sympathetic ganglion (Gaskell's method of dissection). The inhibitory fibres were then stimulated between the ganglion and the brain, and complete inhibition of the heart resulted. The same result was obtained when the heart was being perfused with Ringer's. Similar stimulation when the heart was being perfused with the sodium chloride solution did not cause inhibition of any part of the heart. After change to Ringer's solution stimulation caused complete inhibition.

Experiment 19. — Large frog used. Exposed the intracranial portion of the two vagi, following the technique described in Exp. 18. Before cutting the accelerators they were stimulated, and a distinct acceleration and augmentation was produced by the left, but the stimulation of the right gave no apparent result.

Perfused with Ringer's. Cut the accelerator fibres below the vago-sympathetic ganglion. Stimulated the intracranial portion of the two vagi; the left produced complete inhibition, while the right gave only slight inhibition. The left vagus was used for stimulation in the remainder of the experiment. The sinus and auricle were in perfect rhythm, but the ventricle seemed to be beating independently.

Changed to NaCl 0.7 per cent CaCl_2 0.0115 per cent. There was an immediate increase in tone of heart and also in force of beat. On the NaCl solution which was used before this the rate of beat was 33 before stimulation and 28 during stimulation. On the sodium calcium solution there was complete inhibition during stimulation.

Changed to Ringer's. Heart normal.

Changed to NaCl 0.7 per cent, and the heart became irregular and could not be restored.

It will be noted that the above results of the experiments on the frog are in accord with the observations and conclusions made from our first series of experiments, and corroborate the results of Busquet and Pachon. Irrigation of the frog's heart with normal saline solution removes the cardio-inhibitory function of the vagus for all parts of the heart. But the vagus control of the heart can be restored by irrigation with a sodium calcium solution.

It has been shown by Howell,⁴ that an increase of potassium in the

⁴ HOWELL: *Loc. cit.*

circulating liquid favors this inhibitory action of the vagus nerve on the heart, and this conclusion is corroborated by the present paper in so far as our experiments show that the influence of subminimal amounts of calcium may be brought to the threshold value by the addition of small quantities of potassium chloride to the circulating liquid. Furthermore, Howell and Duke⁵ have demonstrated that one result of the inhibiting action of the vagus is an increased output of potassium from the heart muscle. On the basis of this last fact these authors have proposed the hypothesis that vagus inhibition is at bottom simply a case of potassium inhibition; that is to say, the vagus impulses stop the heart beat by causing the liberation of potassium within the heart muscle, presumably in that portion of the heart, sino-auricular node, in which the heart beat originates. The fact, shown by Busquet and Pachon and corroborated in this paper, that the presence of some calcium is necessary in order that the vagus impulses may inhibit the heart is not, as might at first be supposed, in antagonism to the above hypothesis regarding the part taken by the potassium in cardiac inhibition. It has long been known (Howell,⁶ Locke,⁷ Cushing,⁸ Joseph and Meltzer,⁹ Mines¹⁰ and others) that the motor nerves to the skeletal muscles lose their action in the absence of calcium. Howell and Duke¹¹ have shown that the same fact is probably true in regard to the accelerator nerves of the mammalian heart, and the results of this paper prove simply that this conclusion may be extended to the action of the vagus nerve on the heart. On the basis of these several results one may be permitted to suggest the generalization that the action of nerve fibres on peripheral tissues is possible only in the presence of some calcium as well as sodium in the tissue acted upon. In the presence of normal amounts of potassium and sodium the reduction of the calcium below a certain threshold value destroys the action of the nerve. What part of the neuro-muscular apparatus is paralyzed by the removal of the calcium cannot be deter-

⁵ HOWELL and DUKE: this Journal, 1908, xxi, p. 51.

⁶ HOWELL: Journal of physiology, 1894, xvi, p. 476.

⁷ LOCKE: Centralblatt für Physiologie, 1894, viii, p. 166.

⁸ CUSHING: this Journal, 1902, vi, p. 77.

⁹ JOSEPH and MELTZER: this Journal, 1911, xxix, p. 1.

¹⁰ MINES: *Loc. cit.*

¹¹ HOWELL and DUKE: Journal of physiology, 1906, xxxv, p. 131.

mined from the experiments published; it may be the nerve fibres, the nerve terminals, or the supposed receptive substance in which the latter end. It is, however, sufficiently obvious that the importance of the calcium, so far as the inhibition of the heart is concerned, probably lies in its relations to the production and conveyance of the vagus impulses to the heart muscle rather than to the inhibitory process itself which occurs within the heart muscle. Those who have investigated the relations of calcium to the rhythmic activity of heart muscle are aware that the presence of calcium in physiological amounts never produces inhibition. On the contrary, it causes invariably an increase in tone or in rhythmic contractility. In the absence as well as in the presence of calcium a sufficient excess of potassium will cause complete quiescence of the heart muscle of a nature similar to that produced by the vagus impulses. The results of Busquet and Pachon, therefore, with regard to the relation of calcium to vagus inhibition should not be stated in the form that calcium is necessary to the process of inhibition in the heart, but rather that calcium is necessary in the nerve apparatus for the conveyance of the vagus impulses to the heart muscle.

PERISTALSIS, SEGMENTATION, AND THE MYENTERIC REFLEX.

BY W. B. CANNON.

[From the Laboratory of Physiology in the Harvard Medical School.]

THE neuromuscular structures of the alimentary canal, from the lower œsophagus onward, are singularly uniform. Smooth muscle arranged in an outer longitudinal layer and an inner circular layer, with a primitive nerve plexus between, constitutes in both the stomach and the intestines the machinery by which all the mechanical factors of digestion are managed. The structure of the nerve net in the stomach, so Auerbach reported,¹ is similar to that in the intestines. This view is slightly modified by Dogiel, who states that the ganglia of the net and the individual cell bodies of the neurones are larger in the small intestine than in the stomach and colon.² The axones of the "motor cells" in the plexus, according to Dogiel's description, pass through several ganglia, giving off collaterals to each, before ending in branches to muscle cells. Histological evidence, therefore, indicates a local nervous system fairly typical for all parts of the digestive tube, and so arranged as to correlate the activities of closely neighboring parts.

THE MYENTERIC REFLEX.

The experiments of Nothnagel³ and Lüderitz⁴ showed that stimulation of the small intestine causes contraction above the stimulated

¹ AUERBACH: Amtlicher Bericht über die Versammlung deutscher Naturforscher und Aerzte, Karlsbad, 1862, xxxvii, p. 202. See also Oppel, *Vergleichende mikroskopische Anatomie der Wirbelthiere*, Jena, 1896, i, p. 27; 1897, ii, pp. 124, 471.

² DOGIEL: *Archiv für Anatomie und Entwicklungsgeschichte*, 1899, Supplement-Band, p. 137.

³ NOTHNAGEL: *Archiv für pathologische Anatomie, Physiologie, und klinische Medicin*, 1882, lxxxviii, p. 4.

spot. Basing his judgment on Nothnagel's studies of intussusception, Mall clearly inferred that while a mass in the intestine is arousing contraction above (a contraction which forces the mass downward and thus arouses fresh regions above to contract), active dilatation below is at the same time inviting an easy descent.⁵ The truth of this inference was demonstrated by Bayliss and Starling, whose well-known experiments led them to state, as the "law of the intestine," "Excitation at any point of the gut excites contraction above, inhibition below."⁶ The co-ordinated character of this response, which occurred in the absence of cerebrospinal connections, made them believe that it was a local reflex controlled by Auerbach's plexus — a conclusion which Magnus verified by obtaining evidence of the "law" in excised gut from which Meissner's plexus had been removed.⁷

The method used by Bayliss and Starling in studying the small intestine they applied to the large intestine as well, and found the local reflex present in the colon of both the dog and the rabbit.⁸ That the cat's colon also is the seat of similar co-ordination was discovered by Elliott and Barclay-Smith, who observed that distention of the middle third of the large intestine in this animal causes constriction above the distended area and relaxation below.⁹

By means of a slight modification of the method employed by Magnus I have studied the response of excised portions of all the large divisions of the alimentary canal. The portion to be examined was removed from the body immediately after the animal (cat) was quickly etherized, pithed, and bled, and was placed in oxygenated Ringer's solution kept at body temperature. A silk thread fastened midway in the piece of digestive tract was tied to an L-shaped glass tube (through which the oxygen bubbled), and thus the structure was anchored in the solution. Another silk thread, attached directly opposite the first, connected with a writing lever. The pull of the lever was about 1 gm. The contractions of the muscular ring between the threads

⁴ LÜDERITZ: *Archiv für pathologische Anatomie, Physiologie, und klinische Medicin*, 1889, cxviii, p. 33.

⁵ MALL: *Johns Hopkins Hospital reports*, 1896, i, p. 71.

⁶ BAYLISS and STARLING: *Journal of physiology*, 1899, xxiv, p. 110.

⁷ MAGNUS: *Archiv für die gesammte Physiologie*, 1904, cli, p. 132.

⁸ BAYLISS and STARLING: *Journal of physiology*, 1900, xxvi, p. 107.

⁹ ELLIOTT and BARCLAY-SMITH: *Ibid.*, 1904, xxxi, p. 281.

were thus recorded, and this ring thus acted as an indicator for influences initiated by stimuli applied above and below.

Fig. 1 is a record from a piece of large intestine about 3 cm. from the ileocolic valve. The stimulus was a pinch. When applied below the recording ring, a contraction resulted; when applied the same distance above the ring, not contraction, but slight relaxation, was recorded.

In observations on the œsophagus and stomach the best results

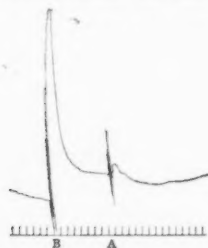


FIGURE 1. — Contraction of ring of colon when stimulus (pinch) was applied below (B); slight relaxation when applied above (A). Time, five-second intervals.



FIGURE 2. — Contraction of ring of œsophagus when stimulus (pinch) was applied below (B, B); slight relaxation when applied above (A). Time, five-second intervals.

were obtained from parts removed three or four days after both vagi had been severed, and the movements of these regions had thus been given time to regain their normal character.¹⁰ Fig. 2 is a record from the lower end of the œsophagus deprived of its remaining vagus nerve supply three days before. Again stimulation by pinching below the recording ring with small forceps caused contraction of the ring, whereas pinching above caused not contraction, but slight relaxation. In Fig. 3 is registered the response of the body of the stomach about 2 cm. below the cardia. The right vagus had been severed nineteen days, and the left five days before. The stimulus was a pinch with forceps applied about 1.5 cm. above, and again the same distance below, the recording ring. The typical contraction occurred when the stimulus was applied below; and no contraction, instead a slight relaxation, occurred when the stimulus was applied above. The same result was obtained when a ring nearer the pylorus was used as an indicator. Since these observations on the stomach were first reported,¹¹ Sick and Tedesko, using chemical in place of mechanical

¹⁰ See CANNON: This Journal, 1906, xvii, p. 442; 1907, xix, p. 438.

¹¹ CANNON: *Ibid.*, 1908, xxi, p. xx.

stimulation, have likewise obtained contraction of the gastric muscle above the stimulated spot, but they usually noted also some contraction below.¹²

From the foregoing evidence it is clear that, by applying to the œsophagus, the stomach, and the colon the method used to show the presence of the local reflex in the small intestine, there can be demonstrated, throughout that part of the digestive tube which is composed of smooth muscle, the presence of an intrinsic arrangement whereby a stimulus causes a contraction above and a relaxation below the point of application. And not only where the tube is open, but also at the sphincters—certainly at the cardiac and pyloric sphincters¹³—is there reason to believe that the mechanism for the local reflex is present.

Magnus proved that in the small intestine the local reflex was due to the functioning of the nerve net lying between the muscular coats. In the new anatomical nomenclature this net is called the "myenteric plexus," and is described as such for the entire alimentary canal. I have suggested, therefore, that the local reflex of the alimentary canal be called the "myenteric reflex."¹⁴



FIGURE 3.—Contraction of ring in body of stomach, when stimulus (pinch) was applied below (B); slight relaxation when applied above (A). Time, five-second intervals.

THE VARIETIES OF PERISTALSIS.

1. In the stomach and colon.—Although there is the foregoing evidence of a mechanism throughout the digestive tube, assuring an

¹² SICK and TEDESKO: *Deutsches Archiv für klinische Medizin*, 1908, xcii, p. 431.

¹³ See CANNON: this *Journal*, 1907, xx, p. 283; and 1908, xxiii, p. 105.

¹⁴ CANNON: *Ibid.*, 1908, xxiii, p. xxvi.

orderly progress of the contents, there is evidence also that this mechanism is not always operative. For example, in the proximal colon and at times in other parts of the large intestine, *antiperistaltic* waves occur quite normally. And in the pyloric part of the stomach, likewise, reversed waves may be seen. These waves, starting from a pulsating ring more tonically contracted than other parts of the tube, move in a direction opposed to that in which the myenteric reflex would be effective. Furthermore normal gastric peristalsis does not require the presence of the reflex mechanism, for the waves sweep from the pulsatile source in an orderly manner to the pylorus after the myenteric plexus has been completely interrupted by a half-dozen incisions encircling the stomach.¹⁵ In the proximal colon, also, downward-running peristaltic waves may be started by a pulsating ring near the ileocolic junction, but the close succession of the waves indicates that any forerunning inhibition must be ineffective or of slight extent, and therefore quite unlike the projected relaxation preceding the moving constriction, so characteristic of the small intestine.¹⁶

2. In the small intestine. — The common presence of the myenteric reflex in the small intestine, and its usual absence or submergence in the stomach and proximal colon, suggest that these regions are fundamentally different. This surmise is supported by the action of nicotine, which abolishes the reflex in the small intestine, but does not in any way disturb either peristalsis of the stomach or antiperistalsis of the colon.¹⁷ Before this evidence is accepted, however, as proving an important difference between the middle and the terminal portions of the digestive tube, the varieties of movement manifested by the small intestine should be examined.

That the small intestine is capable of conducting waves in either direction any one can easily demonstrate by repeating Engelmann's old observation on an animal recently killed.¹⁸ A pinch starts contractions towards the pylorus and towards the ileocolic valve, which sometimes sweep over extensive reaches of the gut. Bayliss and Starling, after coating the surface of an exposed coil with a cocaine solu-

¹⁵ CANNON: this Journal, 1911, xxix, p. 258.

¹⁶ See BAYLISS and STARLING: Journal of physiology, 1899, xxiv, p. 113; 1901, xxvi, p. 138.

¹⁷ See ELLIOTT and BARCLAY-SMITH: *Ibid.*, 1904, xxxi, p. 304; CANNON: this Journal, 1909, xxiii, p. xxvii.

¹⁸ ENGELMANN: Archiv für die gesammte Physiologie, 1871, iv, p. 35.

tion, or injecting nicotine or muscarine, still saw waves of constriction run along the gut, but as often in one direction as in the other.¹⁹ I have removed loops of small intestine from the cat, tied them at each end, filled them with 1 per cent strychnine sulphate, and placed them in oxygenated Ringer's solution kept at body temperature. In one instance I observed repeatedly during a half-hour rings of constriction running along a loop in the normal direction. Then, by pinching the loop midway of its length, I produced a strong contraction which first brought the moving waves to a stop. In about ten minutes, however, the contracted region, having relaxed, was pulsating and sending away waves, in *both* directions. On another occasion a loop thus filled with 1 per cent strychnine sulphate was hung in a moist chamber. The lower end of the loop was dipping in Ringer's solution, to which barium chloride (2:1000) had been added. Oxygen bubbled through the solution and filled the chamber. The part in the solution soon entered a state of high tonic contraction; but after a brief period reversed waves were seen, starting from the region of increased tone and passing upward to the other end of the loop. In both these instances not only were reversed waves observed, as in Engelmann's and in Bayliss and Starling's experiments, but these waves started from a pulsating source. The small intestine, therefore, is capable, under these evidently unnatural conditions, of exhibiting the same sort of activities that are seen in the colon and stomach.

The question now arises, May the peculiar peristaltic mechanism of the small intestine be absent in conditions more natural than those just described? Although the reversed waves described in the above paragraph were probably not preceded by an area of inhibition, it is of interest that several observers have noted, in experiments on the small intestine, an area of inhibition *above* the stimulated spot. Thus Bayliss and Starling found instances of stimulation below a balloon in the gut, causing inhibition of the muscle about the balloon.²⁰ Magnus obtained the same result at times in experimenting on the isolated intestine.²¹ And Langley and Magnus have recorded similar observations.²² In the dog, cat, and rabbit, on which these effects were

¹⁹ BAYLISS and STARLING: *Journal of physiology*, 1899, xxiv, p. 115.

²⁰ BAYLISS and STARLING: *Ibid.*, 1899, xxiv, p. 113.

²¹ MAGNUS: *Archiv für die gesammte Physiologie*, 1904, cii, p. 134.

²² LANGLEY and MAGNUS: *Journal of physiology*, 1905, xxxiii, p. 47.

seen, stimulation of the small intestine, unaffected by drugs, may therefore at times evoke relaxation *above* the stimulated spot. In this connection the testimony of Bayliss and Starling is pertinent, that repeated stimulation of the same piece of gut results in the peristaltic mechanism becoming fatigued. Under these circumstances, they declare, the most striking difference from the normal condition "is the *absence of inhibition* below the bolus."

The discussion thus far suggests that the myenteric reflex does not hold the small intestine in a fixed and rigid mode of action, but may at times be so far in abeyance as to permit waves of constriction to pass in a direction opposite to the normal. This reversal of peristalsis has been seen in the living animal by means of the X-rays. An obstruction was present about 10 cm. beyond the pylorus. The course taken by food passing along the duodenum was carefully traced on transparent paper laid over a fluorescent screen. After the mass which accumulated above the obstruction had been worked over for some time by alternating periods of segmentation and peristalsis, it was suddenly divided, and the proximal portion was moved rapidly back along the course which had been traced, even up to the pylorus. This reversed conveyance of the food was seen repeatedly with perfect clearness.²³ Other evidence that antiperistalsis may occur in the small intestine has been secured by watching directly, some time after operation, the action of a reversed part of the gut. More than three months after operation Kelling thus saw intestinal contents moved towards the colon through a reversed portion by distinct peristaltic waves.²⁴ Similar observations have been reported by Enderlen and Hess, Beer and Eggers, and McClure and Derge.²⁵ And the clinical evidence that in cases of intestinal obstruction continued vomiting of offensive decomposed material occurs after the stomach has been repeatedly washed, can also be interpreted as due to upward-running waves in the intestine. Although, as Mall and others have shown, reversal of a piece of gut is likely to result in the early accumulation of indiges-

²³ CANNON and MURPHY: *Annals of surgery*, 1906, xliii, p. 524. Conceivably the factor of fatigue, which, as already noted, impairs the action of the myenteric reflex, was the occasion for this phenomenon.

²⁴ KELLING: *Archiv für klinische Chirurgie*, 1900, lxii, p. 326.

²⁵ ENDERLEN and HESS: *Deutsche Zeitschrift für Chirurgie*, 1901, lix, p. 240; BEER and EGGERS: *Annals of surgery*, 1907, xlvii, p. 582; MCCLURE and DERGE: *Johns Hopkins Hospital bulletin*, 1907, xviii, p. 473.

tible stuff at the region of the upper suture,²⁶ the results above cited show that in time conditions may arise which alter the functioning of the small intestine *in situ* to such a degree that it can exhibit antiperistalsis.

In recently published papers I have presented evidence that colonic antiperistalsis and gastric peristalsis are responses of the tonically contracted organs to being stretched by the contents.²⁷ The rhythmically repeated waves start at a pulsating tonic ring. They resemble, therefore, the waves seen in excised small intestine filled with strychnine sulphate (see p. 119). And since they are not interfered with by nicotine, they are probably of the same order as the waves which can traverse the small intestine after nicotine has been injected, and also after the establishment of obstruction or reversal. Whether in the stomach, the small intestine, or the colon, these constrictions can move in either direction along the canal.

THE MYENTERIC REFLEX AND THE NATURE OF SEGMENTATION.

Although citing instances of reversal of the usual direction of peristaltic waves in the small intestine, I do not wish to give the impression that normally the waves traverse the gut quite as readily in one direction as in the other. Undoubtedly the ordinary direction for the contents to take is from the stomach onward, because of the action of the myenteric reflex. This reflex requires an extent of several centimetres along the gut for its full operation (see p. 123). As Bayliss and Starling inferred, the correlation of the portions involved in the reflex is probably mediated through paths ascending and descending from the point stimulated.²⁸ On this assumption interruption of these paths by circular incisions through both muscular coats (thus dividing the myenteric plexus) should interfere with peristalsis. In a paper published in 1902 I pointed out that the region just beyond the first attached loop of the duodenum (in the cat) is likely to exhibit rapid

²⁶ MALL: Johns Hopkins Hospital reports, 1896, i, p. 93; SABBATANI and FASOLA: Archives italiennes de biologie, 1900, xxxiv, p. 195; PRUTZ and ELLINGER: Archiv für klinische Chirurgie, 1902, lxxvii, p. 964; 1904, lxxii, p. 415.

²⁷ CANNON: this Journal, 1911, xxix, pp. 238, 250.

²⁸ BAYLISS and STARLING: Journal of physiology, 1899, xxiv, p. 115.

peristalsis.²⁹ In order to test the effect of dividing the myenteric plexus, incisions reaching to the submucous connective tissue were made encircling the gut at intervals varying between 1.5 and 2 cm. throughout the first 45 cm. of the small intestine. (Of course the operation was performed under complete anæsthesia.) After the animal had fully recovered from the operation it was fed 25 c.c. mashed potato with which was mixed 5 gm. subnitrate of bismuth. After the food began to appear in the duodenum, it continued to be present there for two hours of observation. During a half-hour of continuous watching no peristalsis was seen in the region where rapid peristalsis usually occurs. The content of this region, however, was undergoing almost constant segmentation — interrupted now and then momentarily by the excitement of the animal. Later examination confirmed this first experience. The encircling incisions stopped peristalsis, but not segmentation. The ordinary peristalsis of the small intestine, therefore, unlike the peristalsis of the stomach (see p. 118), is seriously interfered with by division of the myenteric plexus.

The foregoing experiment shows, however, that segmentation can continue undisturbed in the presence of numerous interruptions of the myenteric plexus. The inference is justified, therefore, that segmentation is a local response to stimulation — a conclusion which is supported by the rhythmic contraction of any narrow strip of circular intestinal muscle when stretched by the pull of a recording lever. Segmentation can best be explained, indeed, as a local response to distention. Thus Bayliss and Starling observed that rhythmic contractions occurred where the distending balloon in the gut exerted the greatest tension.³⁰ The observations that as a mass of food is being pushed along the intestine the back end is likely to be cut off by a constriction ring, and that in cases of intestinal obstruction the accumulated mass is violently segmented,³¹ both point to distention being the condition for rhythmic contractions. Normal segmentation also is best explained as a response to stretching, for the contraction occurs each time in the bulging region about midway between two previous contractions. Experimental evidence to the same effect I have

²⁹ CANNON: this Journal, 1902, vi, p. 260.

³⁰ BAYLISS and STARLING: Journal of physiology, 1901, xxvi, p. 134.

³¹ See CANNON: this Journal, 1902, vi, p. 261; CANNON and MURPHY: *Loc. cit.*, p. 522.

secured by seizing the active exposed intestine between the fingers at two points a few centimetres apart, and placing the enclosed contents under pressure sufficient to distend the gut. The distention was



FIGURE 4.—A photograph of segmentation, in the small intestine exposed under warm salt solution.

followed by the contraction of a narrow ring of the circular coat; and when the finger pressure was repeated rhythmically—as rapidly as a contracted ring relaxed—a new contraction occurred, not where one had just appeared, but in a fresh region. Now here, now there, the gut responded to the distending contents, a shifting perhaps associated with lessened irritability in the region just recovering from activity.

Because these rhythmic movements were seen after the gut was poisoned with nicotine, cocaine, or muscarine, Bayliss and Starling regarded them as of myogenic origin. In opposition to this conclusion Magnus brought the discovery that so long as intestinal smooth muscle is connected with the myenteric plexus it manifests a refractory period and contracts rhythmically, but when deprived of the plexus it no longer pulsates.³²

The presence in the small intestine of rhythmic segmentation, a local response involving action in the nerve net, is further evidence that the myenteric reflex is not always in control. That these two activities involve a markedly different usage of the neuromusculature of the gut is indicated in Figs. 4 and 5, which reproduce photographs of segmentation and peristalsis, respectively, in loops of small intestine exposed under warm normal salt solution. The contraction that occurs in rhythmic segmentation is narrow, involving in each instance hardly a centimetre of the circular coat; whereas the contraction



FIGURE 5.—A photograph of peristalsis, in the small intestine exposed under warm salt solution.

³² MAGNUS: *Archiv für die gesammte Physiologie*, 1904, ciii, pp. 531, 536. For further discussion see MAGNUS: *Ergebnisse der Physiologie*, 1905, vii, p. 45.

that occurs in peristalsis extends along the canal for 4 or 5 cm.³³ A much larger number of circular fibres are evidently called into service by the myenteric reflex to push food through the canal than are active in any single segmenting contraction. And, furthermore, the area of inhibition demonstrable in peristalsis does not exist in segmentation.

On the basis of evidence now in hand the assumption is reasonable that the rhythmic contractions of segmentation are governed by local motor centres, and that in the operation of the myenteric reflex the augmentor and inhibitory paths consist of neurones which are superintendent in function and which normally affect the subordinate local centres through considerable extents in a positive or negative manner. Dogiel's description of neurones whose axones extend some distance from the site of the cell body and give off collaterals to other neurones in ganglia through which they pass (see p. 114), offers a morphological basis for this conception. And the observation that atropin paralyzes conducting paths but not local centres in sipunculus, whereas cocaine paralyzes the motor centres before stopping conduction, suggests, as Magnus has intimated,³⁴ that the drugs used by Bayliss and Starling (see p. 118), though destructive of the reflex, do not seriously affect the local nerve supply.

THE MYENTERIC REFLEX AND THE VARIETIES OF PERISTALSIS.

The evidence that the myenteric reflex is present throughout the gastrointestinal tract has been given above. Although it can take

³³ BAYLISS and STARLING obtained records showing that the longitudinal and circular coats of the intestine contract simultaneously in any region (see *Journal of physiology*, 1899, xxiv, p. 105). MAGNUS observed that as the small intestine became tonically contracted, it elongated, and he concluded, therefore, that as the circular coat contracts, the longitudinal relaxes (see *Archiv für die gesammte Physiologie*, 1904, cii, p. 137). Quite possibly the change MAGNUS saw was due to the circular coat being thicker and stronger than the longitudinal, for the contraction of the circular coat through a long extent of gut must increase the area of cross-section of the cells laid side by side, and by thus elongating the tube, must stretch the longitudinal coat. I find that if a piece of intestine is cut both *across* and *lengthwise*, and permitted to go into tonic contraction, it takes such shape as permits each of the muscular layers to be in the shortest possible state. In other words, they then contract simultaneously.

³⁴ MAGNUS: *Archiv für experimentelle Pathologie und Pharmakologie*, 1903, I, pp. 97, 103; *Ergebnisse der Physiologie*, 1908, vii, p. 44.

control of the neuromusculature of the canal, it certainly does not always exercise that control. The reflex is most evident in the small intestine, but, as has been shown, it does not govern the rhythmic segmentation which normally occurs in that region, and it may be so completely suppressed as to permit the gut, while still in the intact animal, to exhibit antiperistalsis. We may reasonably assume, therefore, that antiperistalsis of the colon also can occur in the presence of a mechanism which, under certain circumstances, would enforce there the local reflex. And the same assumption may be made regarding the stomach.

What causes the myenteric reflex to appear or not, when material is present, is as yet undetermined. Certainly chyme is not pushed onward continuously from stomach to colon. And yet after a mass of material has lain for some time undisturbed, or has been undergoing segmentation, a peristaltic wave will appear, force the mass onward for some distance, and then stop. If we consider the functions of digestion and absorption which peristalsis subserves, the forwarding of the nutriment might be serviceable, not because the mass as such must be advanced, but because fresh regions for digestion and absorption are needed. The degree of digestion or the status of the mucosa, or some relation between these two, might then explain the peculiarities of peristalsis in the small intestine. Some such regulatory arrangement for the progress of material through the gut is suggested by the results reported by London and his associates. They found that food-stuffs are absorbed at different rates at different parts of the tube — meat most in the upper part, starch and fat most in the lower part³⁵ — and that each portion of the tract, in the case of any particular food, absorbed a constant percentage, quite independent of the amount fed.³⁶ Nutriment when given in small bulk (50 c.c.) was distributed in the small intestine quite as it was when given in large bulk (500 c.c.), so that in either circumstance the entire tract was forced into service.³⁷ Possibly these results are best explained as due to an invoking of the myenteric reflex by the nature of the intestinal contents or the relation between the contents and the mucosa.

³⁵ LONDON and SIVRÉ: *Zeitschrift für physiologische Chemie*, 1909, lx, p. 201.

³⁶ LONDON and SANDBERG: *Ibid.*, 1908, lvi, p. 402.

³⁷ LONDON and DOBROWOLSKAJA: *Ibid.*, 1909, lx, p. 273.

In the colon as well, the nature of the contents may determine whether the myenteric reflex shall take charge of the musculature. In the distal colon, where the contents are more or less hard, onward-moving peristaltic waves prevail. And even in the proximal colon Elliott and Barclay-Smith observed (in the rat) that antiperistaltic waves appeared if the material it received was soft and moist, but that the peristaltic reflex was exhibited when the material was stiff and dry.³⁸

To what extent and under what circumstances the myenteric reflex may assume control of the gastric musculature is not at all known. That it is quite unnecessary for normal gastric peristalsis, I have proved in the experiments already cited in which several complete interruptions of the myenteric plexus had almost no effect on the passage of the waves.

A MORE EXACT TERMINOLOGY.

The foregoing discussion has shown that besides segmentation the alimentary canal manifests several different sorts of moving waves — downward-moving waves with projected inhibition, and downward-moving and upward-moving waves without projected inhibition. All these various forms of undulation have hitherto been termed simply peristalsis. The distinctness of their character, however, justifies distinctive names. The term "peristalsis" means literally, in relation to hollow organs, "encircling contraction." The fact that the contraction occurs around a circular tube renders the retention of *peri* unnecessary. The important facts to be brought out are whether the contraction moves, whether it moves down the tube or up, and whether it is preceded by an area of inhibition. The downward-moving contraction is properly designated as *katastalsis*. The upward- or backward-moving contraction is properly designated as *anastalsis*. Both anastalsis and katastalsis may occur, as stated above, in any portion of the gastrointestinal tract. The katastaltic and anastaltic waves are likely to recur rhythmically and run in a close series, *i. e.*, with no projected inhibition. The contraction which is preceded by a demonstrable region of inhibition is of different order; it is man-

³⁸ ELLIOTT and BARCLAY-SMITH: *Loc. cit.*, p. 285.

aged by the myenteric reflex, and always moves downward. Since the characteristic feature of this activity is the relaxing or opening of the canal in front of the constriction, I suggest as a term to designate it *diastalsis*, in the sense of expansion or dilation. Although each of these words has been used either in Greek or English in a sense different from that here intended, their significance in relation to the alimentary canal is immediately clear. All three terms with *stalsis* in their composition preserve an association with wave-motion along the alimentary canal. The prefix in each case is significant of the peculiarity of the wave motion which is designated.

Of the four mechanical activities of the alimentary canal, then, rhythmic segmentation occurs in the small intestine, *katastalsis* occurs typically in the stomach, and *anastalsis* occurs typically in the proximal colon. Both *katastalsis* and *anastalsis* can occur with segmentation in the small intestine. These three activities are independent of the myenteric reflex. The fourth activity, *diastalsis*, is the resultant of the local reflex in the wall of the canal, and is characterized by an area of inhibition preceding the moving constriction.

SUMMARY.

The myenteric plexus is fairly uniform in structure throughout that part of the alimentary canal which is provided with smooth muscle. Excised parts of œsophagus, stomach, and colon, like the gastric sphincters and the small intestine, exhibit contraction above, and relaxation below, a stimulated point. This may properly be called the *myenteric reflex*.

The myenteric reflex is not always operative. In the stomach and colon it is not operative when antiperistaltic waves are passing, nor is it needed for normal gastric peristalsis. In the small intestine, likewise, the reflex is not in control whenever that part of the canal is manifesting antiperistalsis, as it may do after death, after being treated with certain drugs, and after continued obstruction or reversal. Furthermore, the reflex does not govern rhythmic segmentation in the small intestine.

Evidence is given that segmentation is a local response to internal pressure. Probably in the usual (reflex) peristalsis of the small intes-

tine the same neuromusculature is involved as in segmentation, but subject to superintending and co-ordinating neurons.

What causes the myenteric reflex to occupy at times the local neuromusculature is not known; there is some reason for thinking that the nature of the contents or the relation of the contents to the mucosa determines the appearance of the reflex.

Three terms are suggested to designate the distinctive waves of the alimentary tract: *katalsis*, for downward-moving waves, *analsis*, for upward-moving waves, both usually appearing rhythmically, and not preceded by an area of inhibition; and *diastalsis*, for the downward-moving wave, controlled by the myenteric reflex, and preceded by inhibition.

ATTEMPTS TO PRODUCE EXPERIMENTAL HYPERTHYROIDISM IN MAMMALS AND BIRDS.

By A. J. CARLSON, J. R. ROOKS, AND J. F. MCKIE.

[From the Hull Physiological Laboratory of the University of Chicago.]

I. INTRODUCTION.

THE primary aim in this attempt to produce hyperthyroidism was the establishment of tests for the thyroid secretions in the body fluids. It is clear that such tests might be more readily worked out if we were able experimentally to increase greatly the thyroid secretion in the body fluids. We have as yet no test that undoubtedly demonstrates the thyroid secretions in the blood and the lymph. The well-known aceto-nitrile test of Hunt is a delicate test for some substances in the thyroid gland, but it is questionable whether it is a test for the actual thyroid secretion as passed into the body fluids.¹ Asher's recent experiment on the excitability of the depressor centre has not been sufficiently controlled to be used as a test for the thyroid secretion in the blood.² The biological test for the thyroid secretion under investigation in this laboratory is *blood transfusion in experimental cretinism*; and we reason that the greater the concentration of the thyroid secretion in the blood the less quantity of blood and the less frequent the transfusions necessary to establish or maintain normal growth under conditions of experimental "hypothyroidism" in the young.

What are the physiological criteria of hyperthyroidism? It is generally, though not unanimously, held that Graves' disease or exophthalmic goitre in man is primarily a condition of hyperthyroidism. The cardinal symptoms of this malady are: (1) tachycardia; (2) hyperexcitability; (3) rapid loss of body weight; (4) disturbance of

¹ CARLSON and WOELFEL: this Journal, 1910, xxvi, p. 32; OLDS: *Ibid.*, p. 354.

² ASHER and FLACK: *Zeitschrift für Biologie*, 1910, lv, p. 83.

the digestive tract (diarrhea); (5) exophthalmus. If it were established that exophthalmic goitre is primarily hyperthyroidism, the above symptoms would constitute the desired criterion at least for man. The basis for the prevalent view as to the etiology of exophthalmic goitre is (1) the structural changes in the thyroids, (2) the effects of partial extirpation of the gland, (3) the aggravation of the symptoms by thyroid administration; (4) and the alleged production of some or all of the symptoms in healthy individuals and experimental animals by thyroid administration.

As regards the structural changes in the thyroid glands, their physiological significance is as yet a matter of inference rather than direct demonstration. These changes may signify an *altered* secretion rather than an *excessive* secretion. The changes are very variable. There may be in man considerable hyperplasia of the thyroid without any other evidence of "toxic" goitre; or there may be strong symptoms of exophthalmic goitre with little or no hyperplasia and increase in volume of the glands. The existence, simultaneously, of symptoms of exophthalmic goitre and of myxedema or hypothyroidism in the same individual is another fact that is difficult to bring in line with the prevalent views. This fact alone seems to show that we are dealing with an altered thyroid secretion, or with a number of thyroid secretions that may vary independently of one another, or that some of the so-called cardinal symptoms of exophthalmic goitre are secondary effects of some disturbance of metabolism, and not primarily the result of the thyroid changes — a view ably championed by Marine.³ Moreover, a considerable percentage of the dogs in certain regions of America exhibit the thyroid hyperplasia considered typical of exophthalmic goitre in man, without showing any of the other symptoms of this disease syndrome.⁴ The temporary, or in rare instances permanent, effects of partial removal of the thyroids in exophthalmic goitre are just as readily accounted for on the theory of altered secretion as on the theory of excessive secretion, if they are not instances of spontaneous recovery.

The alleged aggravation of the goitre symptoms by thyroid administration is also capable of a different interpretation. It is obvious that in this disease the organism is less resistant to many unfavorable

³ MARINE and LENHART: Archives of internal medicine, 1911, viii, p. 265.

⁴ CARLSON and WOELFEL: this Journal, 1910, xxvi, p. 32.

conditions than is the normal individual. Even the healthy man appears to be much more susceptible to the toxic effects of thyroid administration than any other mammal. Is it surprising that the weakened exophthalmic goitre patient should be even more susceptible than the normal individual? There is nothing necessarily specific in that fact, because nervousness, tachycardia, etc. are aggravated by many untoward conditions, particularly disturbances of the digestion.

The case for *experimental hyperthyroidism* is best stated — and refuted — by a brief reference to some of the typical investigations in this field. Schoendorff⁵ fed one dog weighing 25 K. with 1.5 gm. to 10 gm. desiccated thyroid per day for about five months. During this period there was gradual loss of weight from 25 K. to 15 K. The urine examination showed that the loss of weight was primarily due to the oxidation of the body fat, but there was also a minus nitrogen balance. Georgiewski⁶ fed from 50 gm. to 100 gm. fresh ox thyroid per day to three dogs, weighing 7.4 K., 2.2 K., and 6.2 K. respectively. Dog I died after five and a half days, having lost 66 per cent of his body weight. Dog II died in eighteen days after losing 34 per cent of his initial weight. Dog III died in fifty-four days, having lost 42 per cent of his weight. The dogs developed tachycardia (pulse up to 200 per minute), polyuria, diabetes, and, in the early part of the experiment, an increased appetite. Similar results were obtained by feeding ox *thymus* to two dogs and one rabbit. The same investigator reports on subcutaneous injection of pressure fluid of fresh ox thyroids into dogs. All of the eight dogs used developed tachycardia, polyuria, diabetes, and emaciation. Tetany and exophthalmus were not observed. Berkeley⁷ fed 5 to 15 grains (fresh gland) of sheep's thyroid to mice (5) and guinea pigs (3). These animals died in from three to ten days. The symptoms developed were emaciation, rapid respiration, tremors, and — myxedema! Cunningham⁸ reports three series of investigations in hyperthyroidism. Series I. Subcutaneous injections of thyroid extracts (sheep, some of these were sterile) into dogs and rabbits. Toxic symptoms re-

⁵ SCHOENDORFF: Archiv für die gesammte Physiologie, 1897, lxvii, p. 395.

⁶ GEORGIEWSKI: Zeitschrift für klinische Medizin, 1897, xxxiii, p. 153.

⁷ BERKELEY: Johns Hopkins Hospital Bulletin, 1897, viii, p. 137.

⁸ CUNNINGHAM: Journal of experimental medicine, 1898, iii, p. 147.

sembling some of those in exophthalmic goitre were produced. *But the same results were obtained by the injections of nucleoproteins or the extracts of other glands.* Series II. Feeding fresh sheep's thyroid to chickens (1), rabbits (4), dogs (3), cats (1), monkeys (4), man (2). *From 40 to 300 gm. were fed daily for fourteen to twenty days without producing any toxic symptoms in any of the animal groups.* Series III. Feeding of desiccated thyroid and stale thyroid. Toxic symptoms were produced in roosters, rabbits, monkeys, and man, but not in dogs. The quantities fed or the length of the feeding experiment on the dogs is not stated. The monkeys weighing about 1.5 K. developed diarrhea, tachycardia, increased excitability, loss of weight, and fever, after receiving from 3 gm. to 4 gm. desiccated thyroid. In man 1 gm. desiccated thyroid per day caused diarrhea, restlessness, cardiac acceleration, and loss of weight. *Feeding experiments with desiccated thymus gave the same results.* Cunningham concludes that the toxic symptoms are due to decomposition products and are not specific *thyroid* effects, as they are not caused by the fresh thyroid glands. Schultz⁹ studied the metabolism of normal and of thyroidectomized dogs fed daily for about three months with desiccated thyroids, representing 2 gm. to 2.5 gm. fresh glands. The normal dogs showed no change in metabolism, but in the thyroidectomized dogs there was an increase in the nitrogen and the phosphorus elimination. Underhill and Saiki¹⁰ fed dogs desiccated sheep's thyroid (Armour) in quantities from 5 gm. to 50 gm. per day from one to ten days. *There was slight, if any, increase in the nitrogen elimination, and only a slight change in nitrogen constituents of the urine.*

The above suffices to show that *undoubted exophthalmic goitre or hyperthyroidism* has not yet been produced in experimental animals by any form of thyroid administration. The experimental results are too contradictory. This may be due in part to differences in the thyroid preparations used; but our own studies indicate that lack of sufficient control of some of the experimental conditions is also a factor. As regards man, the literature seems fairly unanimous on the point that thyroid administration increases oxidation and nitrogen elimination in cretinism and myxedema as well as in normal individuals,¹¹

⁹ SCHULTZ: Festschrift für Rosenthal, Leipzig, 1906, i, p. 291.

¹⁰ UNDERHILL and SAIKI: Journal of biological chemistry, 1908, v, p. 225.

¹¹ MAGNUS-LEVY: Van Norden's Handbuch der Pathologie des Stoffwechsels, 1907, ii, p. 312.

and that excessive thyroid feeding leads to a series of nervous symptoms, more or less similar to those of exophthalmic goitre.

II. METHODS.

The results of thyroid feeding in myxedema and cretinism (clinical and experimental) seem to show that the thyroid secretion is absorbed in active form from the digestive tract. This secretion must therefore be relatively stable. The absorption of the active thyroid substance from the intestine is not surprising in view of the recent findings of Wells and Osborne,¹² that in the guinea pig a number of proteins are absorbed from the digestive tract sufficiently intact or unchanged to produce anaphylactic sensitization. Inasmuch as the secretion seems to be absorbed in its active form from the digestive tract, thyroid feeding is obviously the simplest and the least objectionable method of attempting to induce extra quantities of the secretion in the blood.

The material used by us was Armour's desiccated sheep's thyroid (powdered). We used the whole thyroid substance rather than the isolated and supposedly active thyroid elements (thyroglobulin, thyroid nucleoprotein, etc.) partly because of the difficulty of securing enough of the latter material for an investigation of the present scope, but mainly because it is as yet doubtful whether these substances constitute the thyroid secretion.

The feeding method is open to the objection that while the thyroid secretion is absorbed as such from the intestines the normal animal may have such regulatory mechanisms that the secretion thus absorbed is quickly excreted, altered, or stored so that there is no excess of it in the body fluids at any one time and hence no hyperthyroidism. Negative results by the feeding method are therefore not conclusive. The method of hypodermic injection of thyroid preparations is also open to this objection, but it would not apply to the method of intravenous injection of thyroid extracts. But these two methods are at present open to fatal objections in other directions. All investigators will probably agree that the thyroid secretions have not yet been isolated in pure form. Thyroid pressure fluid, thyroid extract,

¹² WELLS and OSBORNE: *Journal of infectious diseases*, 1911, viii, p. 66; WELLS: *Ibid.*, 1911, ix, p. 147.

thyroidine, etc. are therefore certain to contain many substances not passed into the blood and lymph by the normal thyroid gland. Some of these substances are toxic protein and protein derivatives. The introduction of these directly into the blood will in all probability lead to various symptoms of toxemia, anaphylaxis, etc., quite independent of thyroid secretions. While this was not clear to the early workers on the thyroid, it is so obvious to-day that we must set aside not only these methods but also the results secured with these methods by the earlier workers.

As determined by the method of Oswald, the iodine content of the various lots of Armour's desiccated sheep's thyroid used in this work varied from 0.8 mg. to 1.3 mg. per gram of dried substance.

The effect of the thyroid feeding on digestion and nutrition as determined by the body weight, the appetite, and the nature of the feces can be fairly accurately recorded in all the animal groups employed. The rate and character of the pulse could not be recorded in the birds and in the smaller mammals, and as the pulse record would be of no value in the larger mammals such as monkeys that were more or less wild and struggled violently on being handled, it was also discarded in these groups. Thus the only groups that yielded acceptable pulse records were man, dog, cat, and fox.

An accurate objective criterion for an increased nervous excitability in mammals (excepting man) and birds is wanting. The pulse would be of great assistance on this point. We are familiar with and can recognize the signs of increased nervousness in exophthalmic goitre in the human and the type of hyperexcitability following parathyroidectomy in cats and dogs, but we do not know the expressions of various degrees of nervousness in tame ducks, chickens, rats, or guinea pigs. With the exception of man, dog, fox, and cat, the notes as to the presence or absence of increased nervous excitability in the experimental animals signify merely the presence or absence of restlessness.

The presence or absence of exophthalmus is also largely a matter of personal opinion, unless it is very marked. No observer can fail to recognize a case of pronounced exophthalmus in man. But there are all degrees between the normal appearance and position of the eyeball and extreme exophthalmus. We have been unable to devise any system of measurements that will show slight variations in the position of the eyeballs and the nictitating membranes in the animal groups

under investigation. We had to be content with direct inspection, making the comparison before and during the feeding in the same animal, as well as controlling the fed groups with groups not fed. Some of our colleagues in the laboratory who were not familiar with the fed and the control groups also assisted in making these observations. Nevertheless, there is too great a margin for mere personal opinion. The appearance and position of the eyeballs may seem to vary in consequence of a progressive emaciation. And it is well known that the degree of prominence of the eyeballs varies in different breeds of dogs.

We are aware that our tests should have been completed by taking at least the nitrogen balance of all the fed animals, but we were not in position to carry out this phase of the work.

III. RESULTS.

1. Pigeons.—Our experiments on pigeons were made in three series, (A, B, C) with daily feeding of 0.33 gm., 1.0 gm., and 2.0 gm. desiccated sheep's thyroid respectively. The pigeons were fed by the capsule method.¹³ They offered little or no resistance to the process. Healthy birds of fairly uniform size and age were selected. Besides the thyroid the pigeons were liberally supplied with the routine food. The birds were confined indoors, but they had been accustomed to this mode of living for two months or more before the thyroid feeding began. The results are summarized in Table I.

Series A. Plumage.—In all the birds of this series more or less rapid loss of the feathers began eight to ten days after the beginning of thyroid feeding. In two cases the moulting was so rapid that the birds became practically devoid of feathers for a few days. The feathers of the new plumage did not seem to be of normal gloss and smoothness. It seems that this moulting was caused by the thyroid feeding.

Body weight.—There was a general loss of weight during the first twenty to thirty days of thyroid feeding. This was followed by a return towards the initial weight up to the end of the experiment. The greatest loss in weight appears simultaneously with the period of

¹³ The powdered thyroid was packed in gelatin capsules of suitable size, the capsules moistened and placed on the back part of the tongue, and manipulated until swallowed.

TABLE I.

PIGEONS. SUMMARY OF RECORDS OF BODY WEIGHT ON DAILY FEEDING OF DESICCATED SHEEP'S THYROID. THERE IS AN INTERVAL OF THIRTY-FIVE DAYS BETWEEN THE FIRST (a) AND THE SECOND (b) FEEDING PERIODS OF PIGEONS XVIII AND XIX.

Thyroid per day.	Pigeon.	Body weight, gm.		No. of feeding days.
		Beginning of experiment.	End, or death (*).	
gm. 0.33	I	278	256	60
0.33	II	398	370	60
0.33	III	286	274	60
0.33	IV	325	232	35 (*)
0.33	V	329	205	36 (*)
0.33	VI	335	240	15 (*)
0.33	VII	291	288	60
1.0	VIII	400	300	28 (*)
1.0	IX	430	250	8 (*)
1.0	X	380	255	8 (*)
2.0	XI	350	250	9 (*)
2.0	XII	330	240	7 (*)
2.0	XIII	400	280	7 (*)
2.0	XIV	280	180	4 (*)
2.0	XV	350	250	5 (*)
2.0	XVI	400	260	4 (*)
2.0	XVII	300	220	4 (*)
2.0	{ XVIII a	300	200	4
	{ XVIII b	280	200	8 (*)
2.0	{ XIX a	380	225	4
	{ XIX b	380	250	7 (*)

* Bird died on last feeding day.

rapid moulting, and may be due to the greater rate of oxidation necessary to maintain the temperature of the partly featherless birds rather than to a direct effect of the thyroid feeding on metabolism. This explanation is suggested by the return towards the initial weight on receiving the new plumage. It is also possible that an acquired tolerance to the thyroid feeding is a factor in this recovery of the body weight.

General conditions. — During the rapid moulting the birds were less active and appeared somewhat depressed, but the pigeons that died (Nos. IV–VI) did not seem more depressed on the day before found dead than did those who survived in good condition till the end of the experiment. There appeared to be no increase in excitability at any stage of the experiments. The appetite remained practically normal.

Post-mortem findings. — The post-mortem examination of Pigeons IV–VI revealed no obvious lessons. The emaciation was marked, as these birds had lost one third of their weight.

Series B and C. Plumage. — There was no tendency to moulting or loss of feathers in the birds of these series, despite the large doses of thyroid fed. The moulting observed in Series A must therefore have been correlated with the season (spring) quite as much as with the thyroid feeding.

Body weight. — There is a rapid loss of weight, all the birds having lost about a third of their body weight at the time of death. The rate of the loss per day with 2 gm. per day is only slightly greater than that following 1 gm. per day.

General conditions. — Most of the birds showed symptoms of weakness and depression one or more days before death. No increased excitability or restlessness was observed. Some of the birds ceased eating twenty-four hours or more before death, others continued to eat to within a few hours of death, so that the post-mortem revealed the crop filled with fresh food. All the birds of this series developed diarrhea. This was probably an important factor in the loss of weight.

Post-mortem findings. — All the birds showed hyperemia and hemorrhagic infiltration of the intestinal mucosa, and in three cases this extended to the stomach and the crop. This condition indicates severe digestive disturbance and consequent toxemia.

Summary of experiments on pigeons. — 1. The effect of daily feeding of thyroid in doses of about 1 gm. per kilo body weight seems to

depend on the individual resistance of the birds, about 60 per cent in our series being but little affected, while 40 per cent lost weight and died in twenty to thirty days. Larger doses of thyroid have injurious effects on all the birds (Series B). The symptoms are: loss of weight, diarrhea, weakness, and depression. Appetite may continue to within a day before death.

2. On cessation of the thyroid feeding the birds make apparently complete recovery, even when the excess thyroid administration has been carried almost to the point of death. On resuming thyroid feeding in these birds there appears to be an increased tolerance.

3. The general symptoms together with the post-mortem findings indicate that the thyroid feeding in doses greater than 1 gm. per kilo body weight results in disarrangement of digestion with attendant diarrhea and toxemia.

2. *Chickens.*—A lot of six roosters varying in weight from 2.3 K. to 3.5 K. were selected. Five of these were fed daily Armour's sheep thyroid in capsules. The sixth, or control, rooster received daily similar capsules filled with starch. The roosters offered very little resistance to the feeding process, the thyroid capsules being readily swallowed when placed on the back of the tongue.

Records were kept of the general conditions, the appetite, the plumage, and the body weight. No attempt was made to study the pulse.

Rooster I. — Died May 30 without showing any symptoms prior to May 29, when he appeared dull and depressed. Post-mortem examination revealed some congestion of the viscera.

Rooster II. — Died July 21. There were no obvious symptoms before this date. Post-mortem examination gave no indication of the cause of death.

Rooster III. — Died August 16. This rooster showed some weakness for eight days before death. He was lying down frequently during the day, panting occasionally. Post-mortem examination showed congestion of the viscera and there was some indication of septicemia.

Rooster IV. — Died August 16. Three days before death this rooster showed occasionally a rapid and labored respiration. Post-mortem examination gave indications of septicemia.

Rooster V. — Died July 29. No symptoms prior to death, and post-mortem examination gave no indication of the cause of death.

Rooster VI. — Control. Weight, May, 16 2.65 K.; August 16, 3.20 K.

The animal was kept in the same coop with the rest of the roosters and therefore under the same conditions. He was fed daily starch-filled capsules to the same number as the thyroid-fed animals, in order to determine whether the feeding process itself had any deleterious action. On August 17, we began to feed this rooster 7.5 gm. thyroid per day with the results shown in Table II, No. VI *b*.

TABLE II.

CHICKENS. SUMMARY OF RECORDS OF BODY WEIGHT ON DAILY THYROID FEEDING.

Thyroid per day.	Rooster.	Body weight.		No. of feeding days.
		Beginning of experiment.	At death.	
gm.		kilos.	kilos.	
0.5	I	2.55	2.00	16
0.5-4.0	II	2.60	2.20	66
0.5-6.5	III	2.30	2.00	92
0.5-6.5	IV	3.50	3.00	92
0.5-5.0	V	2.50	3.00	73
<i>Control period.</i>	VI <i>a</i>	2.65	3.20	92
7.5	VI <i>b</i>	3.20	2.50	10

Rooster I must in all probability be excluded in considering the results of this series, as he died on the third day after beginning the experiment, when the quantity of the thyroid fed was so small that it seemed to have no effect whatever on the rest of the animals. That leaves Nos. II-V and the control rooster; in all, five animals. There can be little doubt that these five roosters died from the effects of the thyroid feeding, directly or indirectly.

Effects on the body weight. — There is a rapid loss of weight in No. VI on the large thyroid doses, a slight loss of weight in Nos. II and IV. No. III remained stationary, while No. V gained in weight proportionally as much as No. IV lost. It would therefore seem that death from thyroid feeding may be produced in chickens without any attendant loss of body weight, or with an actual increase in body weight provided the feeding is begun with relatively small doses; while large doses of thyroid probably invariably cause loss of body weight before death.

Plumage. — Only in one case (No. III) was there any noticeable loss of feathers.

General conditions. — At no time was there any increase in excitability that could be detected in their general behavior. There were usually weakness, depression, and diminished appetite for a day or more before death.

Post-mortem findings. — The most constant condition was hyperemia of the viscera, particularly of the intestines. Nos. III and IV gave indications of septicemia. This may have been a secondary result due to disordered digestion from the thyroid feeding.

3. *Ducks.* — Five ducks were secured, three males and two females, apparently full grown, and nearly of equal size and weight. No difficulty was experienced in feeding the thyroid capsules to the ducks. No facilities were available to keep the ducks under the best hygienic conditions. They had to be kept indoors and rather confined, with no facilities for swimming. This fact must be kept in mind in interpreting the results of our experiments, because the slight but gradual loss in weight of Ducks I-IV from April 6 to August 6 is probably due to the confined conditions rather than to the thyroid feeding. Unfortunately, no control bird was kept to settle the point definitely.

During the period from April 6 to August 6, during which time the thyroid fed daily was gradually increased from 0.5 gm. to 6 gm. or from 0.15 gm. to 3 gm. per kilo body weight, no changes in the ducks were noted except the slight gradual loss of weight in Nos. I-IV. The plumage was usually not so smooth and clear as in ducks kept outdoors with plenty of water, but this was probably due to the confined condition. And we are inclined to attribute the slight but gradual loss of weight in Nos. I-IV to the same cause, rather than to the thyroid feeding. On August 6 the ducks were to all appearance normal birds, having at no time exhibited either increased excitability or depression symptoms. The appetite appeared to be normal. No attempt was made to record the pulse.

Body weight. — On August 6 the experiment in group was discontinued, and increasing doses of thyroid given daily to one animal at a time until the death of the animal. With the exception of Duck III, there was no loss of body weight even with doses of 10 to 20 gm. of thyroid per day — 5 gm. to 10 gm. per kilo body weight — doses that speedily killed the ducks.

Plumage. — There was no loss of feathers or other marked change in the plumage.

General conditions. — There was neither marked depression nor increased excitability. The ducks would appear normal in the evening and be found dead in the morning, or in good condition at eight o'clock in the morning and dead by noon.

TABLE III.

DUCKS. SUMMARY OF RECORDS OF BODY WEIGHT ON DAILY THYROID FEEDING. BETWEEN THE FIRST (a) AND SECOND (b) FEEDING PERIODS OF DUCKS I, II, AND III THERE ARE INTERVALS OF FIFTY-SIX DAYS, FIFTY-FIVE DAYS, AND SIXTY-THREE DAYS, RESPECTIVELY.

Thyroid per day.	Duck.	Body weight.		No. of feeding days.
		At beginning of experiment.	At end or death (*).	
gm.		kilos.	kilos.	
0.5-10.0	I a	1.9	1.6	147
10-16	I b	1.6	1.5 (*)	20
0.5-6.5	II a	2.2	1.6	122
10	II b	1.45	1.45 (*)	8
0.5-6.5	III a	2.0	1.80	122
10-16	III b	1.70	1.55	17
0.5-6.5	IV	1.95	1.70	122
0.5-20	V	1.95	2.10 (*)	177

Post-mortem findings. — Duck I, no gross lesions of any kind detected except slight hyperemia of the intestinal mucosa, and an abnormal amount of fluid in the pericardial sack. The crop contained a quantity of food. Duck II, pericardial sack nearly filled with a straw-colored fluid or exudate. The pericardium was hemorrhagic in spots and contained two white (fibrinous) patches. No other lesions found. Duck III, crop filled with food. Hyperemia and hemorrhagic infiltration of spleen, kidneys, and intestinal mucosa. Duck V, no lesions were found.

Summary. — 1. Ducks maintain apparently normal conditions on daily doses of thyroid up to about 4 or 5 gm. per kilo body weight.

2. Daily doses of thyroid in quantities from 5 gm. to 10 gm. per kilo body weight result in the death of the animals in from two to thirty days, without the development of anorexia, emaciation, increased excitability, or change in plumage.

3. The death may be due to the forced feeding with a highly concentrated protein food, desiccated sheep thyroid containing from 12 to 14 per cent nitrogen mostly in the form of protein.

4. *Rabbits and guinea pigs.*—Our experiments were made on nine rabbits and fifteen guinea pigs. The results in the two species are so similar that they can be discussed together. The animals were fed 0.3 gm. desiccated sheep's thyroid per day. The effect of this quantity of thyroid was so rapid and striking that larger doses were not tried. The feeding offered no difficulties. The gelatin capsule containing the thyroid was simply placed in the mouth, and the animal watched till capsule and contents were chewed up and swallowed. As for other food, the animals were given the ordinary routine diet.

Rabbit IV.	{	April 6	1.1 K.
		11	1.0
		14	0.9
		16	0.8 *
		19	0.7 *
		20	0.6 †
Rabbit VII	{	May 5	Weight = 2.00 K.
		10	1.90
		15	1.80
		20	1.75
		25	1.70
		30	1.70
Guinea pig XV	{	June 5	1.75
		8	1.50 †
		10	1.33 §
		May 6	810
		9	620
		11	540 ¶

* Diarrhea. † Died. Congestion of viscera and lungs. Food in the stomach. ‡ Diarrhea, depression. § Diarrhea, depression. Feeding of thyroid stopped. Animal regained weight gradually. ¶ Found dead. Stomach full of food. Congestion of intestine, kidneys hemorrhagic.

We invite attention to the summaries of the results. Three typical experiments suffice to show their general character.

Control guinea pigs. — Records were kept of two pigs picked at random from the same lot, as our experimental animals were kept during

TABLE IV.

RABBITS AND GUINEA PIGS. SUMMARY OF RECORDS OF BODY WEIGHTS ON DAILY THYROID FEEDING.

RABBITS. ¹ AVERAGE BODY WEIGHT AT THE BEGINNING OF THE THYROID FEEDING 1.5 K. QUANTITY OF THYROID PER KILO BODY WEIGHT PER DAY, 0.25 GM.					
No. of animal.	Feeding period. Days.	Percentage loss of body weight at death.	No. of animal.	Feeding period. Days.	Percentage loss of body weight at death.
I . . .	8	33	VI . . .	12	30
II . . .	4	46	VII . . .	36	33
III . . .	33	40	VIII . . .	14	35
IV . . .	14	45	IX . . .	36	43
V . . .	18	45
GUINEA PIGS. ² AVERAGE BODY WEIGHT AT BEGINNING OF THYROID FEEDING, 613 GM. AVERAGE OF THYROID PER KILO BODY WEIGHT PER DAY, 0.60 GM.					
I . . .	13	37	IX . . .	11	33
II . . .	30	50	X . . .	9	25
III . . .	30	51	XI . . .	11	35
IV . . .	4	21	XII . . .	7	32
V . . .	10	30	XIII . . .	6	28
VI . . .	21	46	XIV . . .	9	40
VII . . .	15	28	XV . . .	5	33
VIII . . .	8	42
¹ Average, rabbits				20	41
² Average, guinea pigs				12.4	33

the experiment period in the same cage with them, and given the same diet, with the exception of the thyroid. Both controls *increased* in weight.

Body weight. — There was a rapid and progressive loss of body weight in all cases, the average loss at the time of death being 41 per cent and 33 per cent in the rabbits and the guinea pigs, respectively. This progressive loss of weight and consequent weakness were usually accompanied by normal, or even greater than normal, appetite. Most of the animals at death had quantities of well-masticated food in the stomach. One guinea pig was observed to eat a couple of hours before death, and another one attempted to eat when he was too weak to chew. *The disturbance of digestion was therefore not accompanied by anorexia.*

General conditions. — Diarrhea was practically a constant symptom in the rabbits, but less constant in the guinea pigs. The progressive emaciation was accompanied by depression and weakness, not by increased excitability and restlessness. There was no exophthalmus. No attempt was made to record the pulse, as the heart is too rapid for reliable direct observation.

Post-mortem findings. — The most constant lesions were hyperemia and hemorrhagic conditions of the viscera, particularly the intestines, and the congestion (pneumonia?) of the lungs in the guinea pigs. With one exception the contents of the large intestines and the rectum of both rabbits and guinea pigs was very watery. The diarrhea together with the congestion of the intestines indicates severe digestive disturbances, due directly or indirectly to the thyroid feeding.

The lung infections in the guinea pigs were probably indirect effects of the thyroid feeding, due to a lowered resistance with the progressive emaciation and cachexia.

Our experiments include a sufficient number of animals to prove that rabbits and guinea pigs are severely affected by small quantities of desiccated thyroid *per os*. The progressive emaciation and usual diarrhea remind one of the similar symptoms in exophthalmic goitre in man, but there is no anorexia, nervousness, or exophthalmus. It may also be noted that as the guinea pigs received about twice the quantity of thyroid per day given to the rabbits, as reckoned by body weight in the two groups, the fatal termination of the feeding averaged in the guinea pigs twelve days, in the rabbits twenty days.

5. **Rats.** — The method of feeding consisted in mixing the thyroid powder with cracker dust and water until the mass had the consistency of thick paste. The rats would eat this preparation with great avidity. The quantity of thyroid thus fed per day was 0.33 to 0.36 gm. When it became apparent that this quantity of thyroid had a rapid and deleterious action on the tame or white rats, bred and raised in captivity and accustomed to a mainly vegetarian diet, we extended the experiment to wild or gray rats just captured. The wild rat is omnivorous, if not carnivorous by choice, so that the mere introduction of animal proteins in the diet ought to be less injurious in these animals. It turned out, however, that the wild rats were even more strongly affected by the thyroid than the white rats.

Because of their wildness and viciousness the body weight of the gray rats was recorded only at the beginning of the experiments and at the death of the animals. No attempt was made to study the pulse in either group. The conditions of the appetite, the fur, the feces, and the general activity were recorded daily.

The following typical records may be given as illustrations:

White rat II.	{ April 5	Weight = 80
	10	70
	15	63
	20	58 *
	25	50 †
White rat VIII	{ April 22	Weight = 200
	28	190
	May 10	185
	17	165
	18	155 ‡
Gray (wild) rat II	{ April 21	208
	May 11	120 §

* Diarrhea. † Died. ‡ Died at 5 P. M. Was eating at 8 A. M.
§ Died. Diarrhea for several days before death.

Body weight. — There is progressive emaciation *pari passu* with vigorous appetite until at death the average loss of weight in the two groups was 21 and 34 per cent of the initial weight. The average duration of life under the feeding was twenty-six days in the case of the white and twenty-two days in the case of the gray rats. The average

weight of the wild rats was considerably greater than that of the white, so that the quantity of thyroid per kilo body weight was less in the former than in the latter. Nevertheless the wild rats showed a greater

TABLE V.

SUMMARY OF EXPERIMENTS ON RATS FED 0.36 GM. OF DESICCATED SHEEP THYROID PER DAY.

WHITE RATS. ¹ AVERAGE BODY WEIGHT, 124 GM. THYROID FED PER KILO BODY WEIGHT, 2.88 GM. PER DAY.					
Rat, No.	Feeding period. Days.	Percentage loss of body weight.	Rat, No.	Feeding period. Days.	Percentage loss of body weight.
I	6	19	VII . . .	9	20
II	20	37	VIII . . .	26	22
III	15	15	IX . . .	52	33
IV	6	8	X . . .	50	32
V	33	8	XI . . .	50	15
VI	17	30	XII . . .	24	20
WILD RATS. ² AVERAGE BODY WEIGHT, 158 GM. THYROID FED PER KILO BODY WEIGHT, 2.16 GM. PER DAY.					
I	19	31	V . . .	14	20
II	20	42	VI . . .	24	47
III	14	22	VII . . .	33	30
IV	29	47
¹ Average, white rats				26	21
² Average, wild rats.				22	34

loss of weight and died sooner. This may be due to the fact that the wild rats were not used to the cage confinement rather than to any actual difference in resistance.

General conditions. — As emaciation progressed, there was usually evidence of diarrhea, the soft stools being at times bloody. Despite

this fact the appetite usually continued good to within a day or less than a day of death. With the progress of the cachexia the fur became unkempt and the hair began to fall out. There was no increase in excitability or activity, and no exophthalmus.

Post-mortem findings. — In most cases there was marked congestion of the intestines, and in many cases distinct congestion of the lungs.

TABLE VI.

FOXES. SUMMARY OF RECORDS OF BODY WEIGHTS ON DAILY THYROID FEEDING.

Thyroid per day.	Fox.	Body weight.		No. of feeding days.
		At beginning of experiment.	At end or death (*).	
gm.		kilos.	kilos.	
0.5-2.0	I	3.5	3.0 ¹	67
0.5-1.0	II	4.3	3.7 (*) ²	41
0.5-2.0	III	4.0	3.7 (*) ²	70
0.5-2.0	IV	5.2	4.0 (*) ²	77

¹ Killed by accident. No obvious lesions.

² Post-mortem examination showed throat and lung infection.

The content of the large intestine and the rectum was watery. In two cases there were cysts in the liver, but these probably had nothing to do with the symptoms.

The results on the rats are practically identical with those in the rabbits and the guinea pigs. But the rats are more resistant. In both groups the thyroid feeding leads to emaciation, diarrhea, some loss of hair, lowered resistance to infection, and death. But there is no anorexia, restlessness, or exophthalmus. Unfortunately nothing is known of the effect on the pulse. The post-mortem findings indicate disturbances of digestion and frequently infection.

6. *Foxes.* — We started our experiments April 5, 1910, with a lot of four full-grown healthy foxes, kept in captivity since June, 1909. The foxes proved extremely refractory to the thyroid-feeding process. The animals, although raised in captivity, would not eat when observed. The thyroid could therefore not be mixed with the food. The only other alternative was the placing of the thyroid capsules on the

back part of the tongue so that they were swallowed whole. In the persistent struggle and violent resistance to this process all of the animals were sooner or later injured about the mouth and in the throat, and this led to infection and death, in one case complicated by pneumonia. We are inclined to the view that *the gradual loss of weight of the foxes and the infection and death of three of them within one to two months after the beginning of the thyroid feeding are not the results of the thyroid feeding itself, but of the disturbance and the injuries sustained by the feeding process.* We believe that if the foxes could have been trained to take the thyroid without struggle, they would have shown a resistance to the excess thyroid equal to that of cats and dogs.

7. *Cats.*—Seven healthy cats were selected, four males and three females. Great difficulties were experienced in feeding the thyroid to the cats. The thyroid powder mixed with ground-up meat or with milk was never eaten completely, so we had to resort to feeding the thyroid in gelatin capsules. The cats would not swallow the capsules and contents if they were allowed to chew them up. Consequently we had to select capsules of a size that could be swallowed whole, and these were placed on the back part of the tongue so as not to permit chewing. All the cats struggled violently in the feeding process to begin with, but Nos. II and IV soon became docile and swallowed the capsules voluntarily. The other five continued intractable, and as a result were gradually injured in the throat and mouth; and this led to infection and death in two of them. All of the five refractory cats lost weight during the feeding, but this was in all probability due solely to the struggle, injuries, and shock of the feeding process, and is therefore without significance as regards thyroid physiology. A brief summary of the results on the five refractory cats may nevertheless be presented:

Cat I.—Thyroid fed, April 5 to June 15: 0.5 gm. to 3.0 gm. Body weights: April 5, 4.2 K.; May 5, 4.4 K.; June 5, 3.4 K.; June 15, 4.0 K. Experiment discontinued.

Cat III.—Thyroid fed, April 5 to May 28: 0.5 gm. to 1.5 gm. Body weights: April 5, 5.1 K.; May 5, 5.1 K.; May 25, 4.5 K. Died, May 29, from throat infection and pneumonia.

Cat V.—Thyroid fed, April 5 to June 15, 0.5 gm. to 3.0 gm. Body weights, April 5, 3.4 K.; May 5, 3.4 K.; June 5, 3.7 K.; June 15, 3.0 K. Died, June 17, from throat infection.

Cat VI. — Thyroid fed, April 23 to May 8: 1.5 gm. to 2 gm. Body weights April 23, 4.0 K.; May 7, 2.7 K. Died, May 8, from pneumonia and gangrene of the throat.

Cat VII. — Thyroid fed, May 8, to May 29, 1.0 gm. to 1.5 gm. Body weights, May 8, 3.2 K.; May 19, 3.0 K. Died, May 29, from throat infection and pneumonia.

We now invite attention to the records of Cats II and IV, the only animals of the series that fed without struggle and hence without injury and attendant complications. While the general results on the other five cats are given, we think it is fair to base the conclusions of the experiment on this group on the results of Cats II and IV alone.

TABLE VII.

CAT II. MALE. SUMMARY OF RECORD OF BODY WEIGHT AND PULSE ON DAILY FEEDING OF DESICCATED SHEEP THYROID IN INCREASING QUANTITIES FROM 0.5 GM. TO 18 GM. FEEDING PERIOD, 239 DAYS.

Date.	Thyroid per day.	Body weight.	Pulse.		Average.
			Highest.	Lowest.	
Apr. 5 to Aug. 31	gm. 0.5-8.5	kilos. 3.2-3.7
Sept. 1 to Sept. 20	10.0	3.7-3.3	160	120	130
Sept. 21 to Oct. 24	15.0	3.3-3.4	150	102	123
Oct. 25 to Nov. 30	18.0	3.4-2.0	140	118	122
Dec. 1 to Jan. 21	No thyroid.	2.0-3.8	130	120	123

Cat IV. — Female. Thyroid fed, April 5 to August 31, 0.5 gm. to 7.5 gm. per day. Body weight, April 5, 3.8 K.; May 6, 3.2 K.; June 6, 3.0 K.; July 10, 3.8 K.; August 31, 3.8 K.; The animal gave birth to four kittens on June 15, and was allowed to nurse three of them to August 15. The experiment was discontinued August 31, after a feeding period of 148 days, with the animal in normal condition.

The pulse. — The daily record of the pulse of Cat II from September 1, 1910, to the end of the experiment, January 21, 1911, revealed no change. During this period the rate remained practically constant, and the pulse was at the same time quite regular, much more so than in our dogs. From October 25 to December 1, 1910, when the cat re-

ceived 18 gm. of thyroid per day, the cat lost more than a third of its body weight, yet *no tachycardia developed*.

Body weight. — When desiccated thyroid is fed in quantities of 5 to 6 gm. per kilo body weight per day, there are gradual loss of weight, diminished appetite, a tendency to diarrhea, and a rough and unkempt condition of the fur. This loss in weight is quickly regained on cessation of the thyroid feeding.

General condition. — There was no increased excitability at any stage of the experiments even in the cat receiving 18 gm. of thyroid per day. This cat appeared to continue in practically normal cerebral activity even when he was losing weight rapidly. He would play and purr, and did not seem sick until about November 20, 1910.

The cat is therefore relatively resistant to thyroid feeding. Six grams or more per kilo body weight daily lead to emaciation, diarrhea, and diminished appetite, but no tachycardia or increased excitability or exophthalmus. The clinical pictures of such cats are quite different from typical cases of exophthalmic goitre or "hyperthyroidism" in man.

8. *Dogs.* — The experiment was started April 5, 1910, with a lot of four dogs in normal good condition, ranging in weight from 7 to 9 kilos. We did not foresee the great quantity of desiccated thyroid required to affect dogs, otherwise much smaller specimens would have been selected. Smaller dogs, weighing only 3 to 4 kilos, were selected for the later experiments. The thyroid powder was mixed with moistened ground-up meat, and this was taken by the dogs with great avidity.

In order to make the pulse record of any value, it must be taken under as nearly uniform condition as possible, and, as is well known, this is very difficult to secure in dogs. We adopted the following plan. The pulse record was taken in the morning before feeding, and the dogs were trained to lie down without restraint, while the rate and character of the heart beat were recorded. But even in this way one cannot maintain uniformity in the degree of cerebral emotional activity, owing to the varying degree of playfulness of the dogs from time to time. Dog I especially gave us great trouble on this point. It is obvious that only a relatively great and persistent difference in the rate and character of the heart beat in the dogs can have any significance with regard to specific actions of thyroid secretions. The pulse

record of each dog was taken daily. The variations shown in the summaries given in Table VIII are typical for the entire series.

TABLE VIII.

SUMMARY OF RECORDS OF BODY WEIGHT AND PULSE OF THE SEVEN DOGS FED ON DESICCATED SHEEP'S THYROID.

No. of dog.	Thyroid feeding.		Body weights, K.		Pulse.		
	Quantity per day.	Duration of feeding.	Beginning.	End.	Highest.	Lowest.	Average.
I . .	gm. 0.5-40	days. 239	7.2	7.8	140	78	115
II . .	0.5-20	148	9.1	12.2	126	85	110
III . .	0.5-20	148	8.4	10.3	126	74	100
IV . .	0.5-20	167	7.5	7.5	150	75	120
V . .	20	73	2.9	3.3	124	110	118
VI . .	60	10	3.1	2.8	140	125	135
VII . .	100	17	5.0	3.7	150	125	135

The pulse rate. — Our records show that the heart rate of Dogs I, III, and IV was slightly higher towards the end of the experiments, when the dogs received the largest doses of thyroid. There was practically no change in the pulse rate of Dogs II, V, VI, VII.

The character of the pulse. — We were surprised to find the great irregularity in the heart beat in the majority of apparently normal dogs in our laboratory. The irregularity appears not only in sudden variations in the rate, but in what appears to be an auriculo-ventricular inco-ordination resulting in the occasional "loss" of a beat. This occurs even when the dogs are lying down and are to all appearances calm. But this apparent calmness is probably deceptive, because the cardiac irregularity increases when the dogs become distinctly excited. It is therefore of nervous or reflex origin. This is shown further by the fact that the irregularity disappears when the dogs are put under a general anesthetic. These cardiac irregularities were in evidence in all of our dogs in this series. But they *decreased* as the experiment proceeded. This was evidently due, not to any favorable action on the

heart and the cardio-regulatory nervous mechanism by the thyroid feeding, but to the fact that the dogs became accustomed to the experimental routine, so that the excitement factor was to that extent eliminated.

It can therefore be said that desiccated sheep's thyroid fed to dogs daily in quantities up to 4 to 6 gm. per kilo body weight for long periods has no effect on the regularity of the pulse, and slight, if any, effect on the rate. If the rate is affected, it is in the direction of acceleration. *Tachycardia is therefore not, or at least not readily, produced in dogs by thyroid feeding.*

The body weight. — The results touching this point are practically negative, with the exception of Dog VII, who received 20 gm. per kilo body weight per day. The dogs either maintained their weight or gained in weight. The gain in weight was probably due to normal growth and to improved hygienic condition and care rather than to the thyroid administration.

General conditions. — There was no digestive disturbance (except in Dog VII), diminished appetite, nervousness, restlessness, depression, or change in the eyeballs that might be interpreted as exophthalmus. In short, the dogs continued as normal healthy and happy individuals. This applies also to Dog VII, despite the diarrhea and loss of body weight. The dog remained playful and lively in disposition.

It is clear from the above that none of the supposed symptoms of "hyperthyroidism" or exophthalmic goitre can be produced in normal dogs with daily thyroid feeding in quantities up to 6 gm. per kilo body weight. Daily thyroid feeding in quantities up to 20 gm. per kilo body weight per day leads to digestive disturbances, probably owing to the excessive and concentrated form of the protein thus administered.

Primates. — The work on the primates comprised three rhesus monkeys and one of the authors (A. J. C.). Two of the monkeys were relatively tame, having been used for experimental purposes in the psychological laboratory here for several years. Nevertheless, they were not tame enough to be handled for the purpose of feeding and recording pulse without violent struggling and excitation (fear or anger). Under such conditions the recording of the pulse would not have yielded any reliable data. In all three monkeys before and throughout the course of the feeding, the pulse was too rapid to record accurately by palpation.

Various expedients were tried to induce the monkeys to take the thyroid powder voluntarily. The thyroid was thoroughly mixed with peanut meal or raisins and pressed into a ball, but the animals would

TABLE IX.

DAILY RECORD OF BODY WEIGHT, PULSE, AND GENERAL CONDITION ON FEEDING DESICCATED SHEEP'S THYROID TO AN ADULT HEALTHY MAN. AGE, 36 YEARS.

A. RECORD OF FIRST EXPERIMENT.					
Date.	Thyroid taken.	Body weight.	Pulse.	Temp. of room.	General condition.
	gm.	kilos.			
May 24	1	73.0	65	24
" 25	1	...	66	26
" 26	1	...	75	30
" 27	1	...	80	31
" 28	1	...	72	28
" 29	1	...	70	23
" 30	1	72.4	68	24
" 31	1	...	70	23
June 1	2	...	75	24
" 2	2	...	80	26
" 3	2	...	75	31	Dizziness.
" 4	2	...	78	27	Slight weakness.
" 5	2	72.4	80	24	Dizziness, slight weakness.
" 6	2	...	76	24	Dizziness, slight weakness.
" 7	2	...	78	27	Dizziness, slight weakness.
" 8	2	...	80	28	Dizziness, slight weakness.
" 9	2	...	84	30	Dizziness, slight weakness.
" 10 ¹	2	71.6	80	30	Dizziness, slight weakness.

¹ Experiment discontinued because of dizziness. June 20, body weight, 73.0 K. Conditions normal. July 10, body weight, 73.4 K.

take the prepared morsel, sniff it, and throw it away. Our final recourse was gelatin capsules moistened, placed on the back part of the tongue and manipulated with the finger on a blunt pair of forceps until swallowed. There appeared to be no decrease in the resistance to this mode of feeding throughout the experiment. The monkeys proved as intractable as the foxes.

TABLE X.

B. RECORD OF SECOND EXPERIMENT.				
Date.	Thyroid.	Body weight.	Pulse.	General condition.
	gm.	kilos.		
Oct. 15	..	76.7	80	Normal; 135 c.c. of blood drawn from an arm vein.
" 17	2.0	76.8	75	Normal.
" 18	2.0	...	82	Normal.
" 19	2.0	76.0	90	Headache, weakness, perspiration.
" 20	2.0	75.2	95	Headache, insomnia, weakness, perspiration.
" 21 10 A.M.	2.0	74.6	100	Condition same as on 20.
" 21 10 P.M.	12.0	...	110	Condition same as on 20.
" 22	...	74.0	114	Condition same as on 20; 150 c.c. blood drawn from arm vein.

It is well known that monkeys vomit readily; and our mode of feeding the thyroid might cause regurgitation and loss of the substance. To guard against this error the monkeys were closely watched for ten minutes after the feedings, and the cages scrutinized every day for remnants of the gelatin capsules. In no case was vomiting observed except in the case of Monkeys II and III, on July 3, when an attempt was made to increase the dose to 1 gm. per day by feeding a second 0.5 gm. capsule six hours after the first feeding. In both cases the capsules were regurgitated. It was then decided to continue the dose at 0.5 gm. per day.

The thyroid fed in the two experiments on man was from the same stock used throughout this investigation. It was taken in 0.5 gm. gelatin capsules about thirty minutes before the midday meal. The pulse record and the room temperature were taken at the same hour.

No change was made in the diet or the daily work, except as the work had to be discontinued because of the effects of the feeding.

There was no diarrhea or diminished appetite. Beginning October 19, there was a more or less continuous feeling of tension or distress

TABLE XI.
RESULTS ON THE MONKEYS.

MONKEY I. FED DAILY 0.5 GM. DESICCATED THYROID.		
Date.	Body weight.	Remarks.
April 17	kilos. 2.9
" 19	2.7
" 23	2.4	Diarrhea, weakness.
" 25	2.3	Diarrhea, weakness.
" 28	2.2	Found dead at 8 P. M.
MONKEY II. THYROID FED, JUNE 21-30, 0.33 GM., JULY 1-7, 0.5 GM.		
June 21	kilos. 2.00
" 27	1.90
July 1	1.80	Inco-ordination of head; weakness.
" 5	1.80	Inco-ordination of head; weakness.
" 7	1.70	Feeding discontinued.
July 14	1.90	No weakness and inco-ordination.
MONKEY III. THYROID FED, JUNE 21-30, 0.33 GM. JULY 1-7, 0.5 GM.		
June 21	kilos. 1.90
" 27	1.80
July 1	1.75
" 5	1.65	Weakness; inco-ordination of head.
" 7	1.46	Feeding discontinued.
July 14	1.75	No weakness and inco-ordination.

in the abdominal region, and occasional acute abdominal pains. During October 20-23 the conditions noted were sufficiently severe to render the subject unfit for work. By October 26 the pulse and general conditions had returned to normal. The above experiment was carried out for a different purpose, but the results noted bear directly on the question now under discussion.

Discussion of results. — In the two experiments on man the distinct effects were (1) *loss of weight*, and (2) *dizziness* and weakness, and (3) tachycardia. The loss of weight was too great to be an accidental fluctuation. There was no lack of appetite, no digestive disturbance, or other complications to account for it.

The dizziness and an occasionally slight blurring of vision were undoubtedly an effect of the thyroid feeding, as those symptoms were unknown to the subject prior to, and promptly disappeared on, the cessation of the experiment. At times the dizziness itself was sufficient to interfere with intellectual work.

In the first experiment the pulse record must be considered as negative. A regular pulse that at no time exceeds 84 per minute in warm weather cannot be designated as tachycardia. There was no evidence of nervousness, either subjective or objective. In fact, if this experiment had been run on a dog or a monkey, the only effect that could have been recorded was the slight loss of weight, as the dizziness and disturbance of vision were not great enough to produce objective signs. There can be little doubt, however, that such signs would have been brought about if the thyroid feeding had been continued longer or the dose increased, as was done in the second experiment. The experiments indicate *a relatively high susceptibility of man to thyroid administration per ounce, as compared to other mammals and to birds.*

The symptoms developed by the three monkeys were (1) loss of weight, (2) weakness, (3) diminished appetite, and in one case diarrhea, (4) inco-ordination of the head, probably due to dizziness or weakness. As to the loss of weight, it might be due to diminished appetite as well as to direct action of the thyroid on tissue oxidation. It was undoubtedly due to the thyroid feeding directly, as Monkeys II and III gained in weight on cessation of the experiment. The weakness might be due to the loss of weight. The peculiar bending of the head now to one side, now to the other, and again forward on the chest have not been observed in any other group of animals used in

this investigation. The cause of the phenomena can only be conjectured (weakness, dizziness, pain in the head or ears?). It is difficult to establish an objective criterion for nervousness in monkeys, especially when the pulse cannot be recorded. If activity be taken as criterion,

TABLE XII.

QUANTITY OF DESICCATED SHEEP'S THYROID PER KILO BODY WEIGHTS
PER DAY GIVEN BY MOUTH.

Species.	Tolerated without obvious symptoms.	Causing moderate to severe symptoms and death.
	gm.	gm.
Man	± 0.01	0.03 (?)
Rhesus monkeys . . .	-0.25	0.25
Rabbits	-0.25	0.25
Guinea pigs	-0.50	0.50
Pigeons	± 1.00	1.50-2.00
Chickens	± 1.00	1.50-2.00
Rats	-2.00	2.00
Cats	4.00- 5.00	5.00-6.00
Ducks	5.00- 6.00	6.00-10.00
Foxes	4.00- 5.00(?)	5.00-20.00(?)
Dogs	6.00-10.00	10.00-20.00

the thyroid feeding did not increase this excitability, as the animals became, if anything, more quiescent. But this was probably due to the loss of weight and weakness.

In the light of the effects of thyroid feeding on mammals and birds, the monkeys seem to exhibit about the same resistance as the rodents. They are probably somewhat more resistant than man, but much less resistant than the carnivorous mammals and the birds.

IV. SUMMARY AND CONCLUSIONS.

1. Toxic symptoms can in all probability be produced in all animals by thyroid feeding. The great variations in the resistance to thyroid

feeding in the different animal groups may be re-emphasized by the following summary:

The cause of these variations must remain a matter of conjecture, at least until we know more precisely the nature and the mode of action of the toxic agent in the thyroid. At present we do not know whether the more resistant groups owe their resistance to a greater action of the digestive ferments on the toxic substances, to a diminished absorption, to a rapid destruction and excretion, or to a greater tolerance on the part of the tissues in general.

2. -In the case of the more resistant groups the symptoms produced by the *large* doses of thyroids are probably not specific thyroid effects, but complicated by the effects of the excessive protein diet. This is suggested by the fact that the more resistant groups are almost exclusively carnivorous (dog, cat, fox, duck¹⁴) and are accustomed to a high percentage of animal protein in the food. The fact that the omnivorous and the herbivorous animals are, on the whole, much less resistant suggested the possibility that a part or the whole of the toxic action of the thyroid in these groups is not specific but due to an excess of animal proteins. This hypothesis has been put to the experimental test in this laboratory by Dr. French, the susceptible groups being fed with corresponding quantities of desiccated pancreas, kidney, muscle, and liver of sheep and dog. These organs proved invariably less toxic than the thyroid.

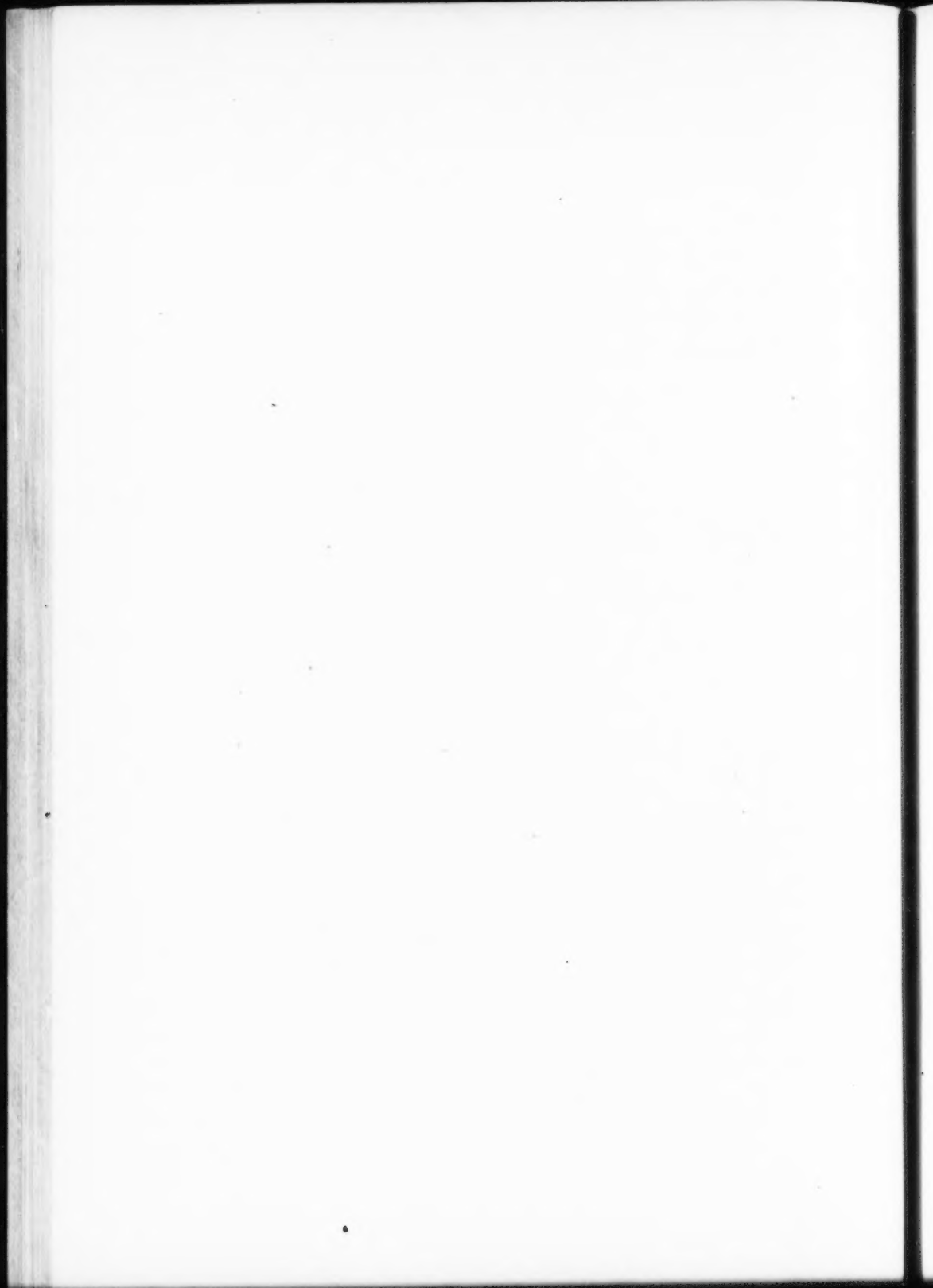
3. When the thyroid is fed in toxic quantities, the most constant symptoms in all the animal groups are *loss of body weight, gastro-enteritis, and diarrhea*. The loss of body weight usually appears and may continue for some time before the enteritis and diarrhea are in evidence. But in some cases, particularly in the ducks, the feeding terminated fatally without any distinct digestive disturbance. In the same way, the feeding may end fatally without any marked loss of weight. This is particularly true for the ducks. It is obvious, however, that in an investigation comprising as many animals as the present one and extending over a long period a certain percentage of the animals will die from causes not directly associated with the thyroid feeding. Undue emphasis should therefore not be given to these exceptions, but the ducks should be further investigated touching this point.

¹⁴ The duck should probably be designated as omnivorous rather than as carnivorous.

4. In the groups in which the pulse could be recorded with accuracy, thyroid feeding in distinctly toxic quantities does not produce tachycardia. Neither does the feeding result in nervousness or exophthalmus in any of the groups. The animals usually retain their appetite and will feed until the gastroenteritis is extreme or the animal is moribund. In view of our results in so many animal groups it would seem that the records of exophthalmus obtained by previous observers by thyroid feeding are based upon errors of judgment, and that the nervousness and tachycardia similarly described are due to some other factors than the thyroid feeding. This is clearly the case in some instances from the accounts of the experiments given by the authors themselves; for example, in Cunningham's experiments on monkeys.

5. It would require considerable imagination or an undue influence of one's wish or one's judgment to identify the symptom complex of excessive thyroid feeding in experimental animals with the exophthalmic goitre syndrome in man. The symptoms in experimental animals may or may not be an expression of hyperthyroidism. Other lines of investigation must determine that point. The symptoms are not those of exophthalmic goitre.

6. It would seem that man is very much more susceptible to thyroid feeding than any other species so far subjected to the experiment. Because of this fact and in view of the higher or more delicately balanced nervous organization in man, the expression of hyperthyroidism in man may differ from that in the lower animals. It is therefore clear that the present study contributes nothing to the settlement of the question of etiology of exophthalmic goitre in man, except in so far as it renders untenable the argument from "experimental hyperthyroidism." Unfortunately, the monkeys are not suited for experiments with thyroid feeding because of their great aversion to thyroid in their food. And as the opportunities to feed thyroid in marked toxic quantities to normal and healthy humans are necessarily rare, this phase of the question must remain unsettled for the present.



RELATION OF THE LIVER TO THE FIBRINOGEN CONTENT OF THE BLOOD.

By WALTER J. MEEK.

[From the Physiological Laboratory of the University of Wisconsin.]

THE earliest work on the origin of fibrinogen was done by Claude Bernard in 1848, Lehmann in 1851-52, and Brown-Séquard in 1869.¹ These authors directed their attention to the distribution of fibrinogen in various veins and arteries. They found the blood from the liver and kidney very poor in this coagulating proteid, while the mesenteric veins were rich. Conclusions were therefore drawn by them and their followers that the intestines produced fibrinogen. Dastre² reviewed this early work and in the main confirmed it. His analyses showed more fibrinogen in arterial blood than in venous. He decided that there must be certain organs which produce and others which destroy fibrinogen. In the first class he placed the skin, lungs, and mucous coat of the intestine; in the second class, the liver and kidneys. This argument was strengthened by analyses showing that the blood from the portal vein was especially rich in fibrinogen. Later workers have criticised the methods used by these earlier authors in determining the amounts of fibrinogen in the blood.

In recent years the origin of fibrinogen has been attributed not only to the intestines but to the bone marrow and the liver. Mathews³ revived the old ideas and concluded from a long series of experiments that fibrinogen was produced in some part of the intestinal area. In seven different animals he found that in all but one, blood from the mesenteric vein was richer in fibrinogen than blood from the carotid. Starting from observations that fibrinogen is regenerated after its removal from the blood, he tested the power of cats to reform the pro-

¹ BROWN-SÉQUARD: *Journal de physiologie*, 1851, i, p. 298.

² DASTRE: *Archives de physiologie*, 1893, v, pp. 686, 169, 327, 628.

³ MATHEWS: *this Journal*, 1899, iii, p. 53.

teid after defibrination and excision of various organs. In this way he decided that the spleen, pancreas, kidneys, lymph glands, and reproductive organs had no part in the reformation. After extirpation of the intestines, however, there was regeneration in only one of five animals, and the amount in this case was very small. In the other four animals the fibrinogen remaining after the defibrination had even disappeared. Mathews' results were somewhat contradictory. Although he found a decrease in fibrinogen after excision of the intestine, he reported three experiments in which the mesenteric veins were ligated, and three other experiments in which the coeliac axis as well as the mesenteric veins were tied, all without any effect on the amount of fibrinogen in the blood. Mathews discovered a relation between suppurating processes and the amount of fibrinogen. From the results obtained by intestinal extirpation and by experimental suppurations he concluded that for the most part fibrinogen was formed by leucocytic decomposition in the intestinal area.

Starting from the clinical observations that the amount of proteid is increased in patients suffering from infectious diseases, Müller⁴ produced infections in guinea pigs and studied the proteid distribution in the blood and bone marrow. He found a marked increase in the fibrinogen content of the bone marrow, and accompanying this was an increase in the number of leucocytes and great lymphoid activity. He therefore concluded that the bone marrow was the chief seat of fibrinogen formation. Morawitz and Rehn⁵ came to a similar conclusion in another way. They whipped the fibrinogen out of the drawn blood, reinjected, and then sought carefully for the tissues showing unusual activity during the period of regeneration. The spleen and especially the bone marrow showed a great number and an active proliferation of myelocytes. On this evidence the authors decided that the bone marrow was a fibrinogen former.

Doyon and Kareff⁶ in 1904 reported a single experiment in which the portal vein of a dog was connected by a parafined cannula to the vena cava by way of the hepatic vein. The lobes of the liver were then excised. The animal lived only nineteen minutes after the com-

⁴ MÜLLER: Beiträge zur chemischen Physiologie, 1905, vi, p. 454.

⁵ MORAWITZ and REHN: Archiv für experimentelle Pathologie und Pharmacologie, 1908, lviii, p. 141.

⁶ DOYON and KAREFF: Comptes rendus de la Société de Biologie, 1904, p. 612.

munication was established, but in that time the blood had become completely non-coagulable. Doyon advanced this rather meagre evidence in support of his contention that the liver was the seat of fibrinogen formation. This hypothesis he further supported by experiments showing the failure of frogs to regenerate fibrinogen after removal of the liver, and by cases of phosphorus and chloroform poisoning in dogs in which the disappearance of fibrinogen proceeded along with lesions in the liver.

Nolf⁷ repeated the work of Doyon on liver extirpation in the dog and failed to confirm it unless very special preliminary preparations were made. He connected the portal to the right auricle by a long cannula and joined the abdominal and thoracic venæ cavæ in the same way. The liver was then entirely removed. Dogs subjected to this procedure lived about two hours without any change being noted in the blood. If however the operation was preceded by heavy meat feeding, or if during the experiment there was injection of peptone or stasis in the intestinal area, the fibrinogen might begin slowly to disappear. In only two cases did the blood ever become completely non-coagulable. Nolf decided that two factors were chiefly responsible for the phenomena observed, namely, fibrinogen production and fibrinogen consumption. He believed that the liver was producing fibrinogen and thrombin. Leucocytes and endothelial cells were at the same time consuming fibrinogen. His operative procedures by extirpating the liver cut off the source of fibrinogen and by injecting peptone stimulated the consumption of it by the leucocytes. Nolf gave no quantitative estimates of fibrinogen at any stage of his experiments.

In a recent paper Whipple and Hurwitz⁸ have carefully studied the effect of chloroform poisoning on the liver and fibrinogen content of the blood. They found that fibrinogen disappeared at a rate exactly corresponding to the amount of liver necrosis, and it regenerated at a rate exactly corresponding to the rapidity of liver repair. They concluded that fibrinogen was either formed in the liver or was wholly dependent on that organ for its production.

A review of the work done on the relation of the liver to fibrinogen formation, as well as suggestions from Dr. Eyster, led us to attack the

⁷ NOLF: Archives internationales de physiologie, 1905, iii, p. 1.

⁸ WHIPPLE and HURWITZ: Journal of experimental medicine, 1911, xiii, p. 136.

problem by means of the Eck fistula. This operation would cause less mutilation of the animal than the methods of Doyon and Nolf. In addition we planned to secure quantitative estimations of the fibrinogen at various stages of the experiments. After some preliminary work on cats in order to develop the technique the following plan was mapped out:

1. To find the normal rate of fibrinogen regeneration after it had been reduced as much as possible in the blood.
2. To find if fibrinogen is regenerated after an Eck fistula.
3. To find the part played in regeneration of fibrinogen by the arterial blood to the liver.

TECHNIQUE.

Dogs were used in all of the experiments reported in this paper. The Eck fistulæ were made after a method described by Fischler and Schröder.⁹ This consisted first in approximating the veins and sewing them together at the lower margin of contact with a single row of stitches. The threads from the uppermost stitch were left long. A cutting thread was next placed in position. This passed into the lumen of the portal, forward 1.5 cm., across into the lumen of the vena cava, back 1.5 cm. and out. The ends of the cutting thread were left quite long. The second row of stitches completing the anastomosis between the veins at the upper margin of contact was next made. After allowing a few minutes for small clots to cement the veins together, the ends of the cutting thread were taken one in either hand and the opening cut through by sawing back and forth with the cutting thread. An assistant held the long ends from the uppermost stitch to support the anastomosis during this procedure. There was usually very little hemorrhage, in fact none if the stitches had been laid carefully. This method is practically the same as that described by Sweet¹⁰ except a silk thread is used for cutting instead of a cautery wire. The method is easier and seems more satisfactory in every way than the old methods in which scissors are used to cut the openings in the veins.

Previous workers have determined the amount of fibrinogen in

⁹ FISCHLER and SCHRÖDER: *Archiv für experimentelle Pathologie and Pharmacologie*, 1909, lxi, p. 428.

¹⁰ SWEET: *Journal of experimental medicine*, 1905, vii, p. 163.

the blood in three different ways. The first method consisted in whipping the fibrinogen from the blood in the form of fibrin. This was ground up and washed in a mortar with water and normal saline until the washings no longer gave a biuret reaction. The fibrin was then caught on the weighed filter through which the washings had been passed, washed free from chlorine, extracted with alcohol and ether, and dried to constant weight.

The French workers as well as some of the Germans have used the method of Reye.¹¹ Reye found the percentage of ammonium sulphate necessary to precipitate fibrinogen from plasma. Blood was first drawn into a 3 per cent sodium fluoride solution until the fluoride was diluted to .5 per cent. To every 10 c.c. of plasma secured after standing or centrifuging, there was added 25 c.c. of distilled water and 13.4 c.c. of a saturated solution of ammonium sulphate. On cooling in the ice chest a flocculent precipitate appeared. This was caught on a weighed filter and washed with a corresponding strength solution of ammonium sulphate until the washings no longer gave a biuret reaction. The filter was then dried at 85° C. for twelve hours. The precipitate of fibrinogen now coagulated by heat was washed with hot water until sulphur free, extracted with alcohol and ether, dried for two hours at 100° C., and placed in the desiccator until constant weight was maintained.

Whipple and Hurwitz¹² coagulated the fibrinogen by heating the plasma from 58° to 60° C. for ten to twelve minutes. The fluoride plasma was first made faintly acid with acetic. After heating the coagulum was centrifugalized down and caught on a weighed filter.

In our experiments we have relied principally on the first method. As carried out by early workers this method was necessarily inexact, since they knew little or nothing about the nature of thrombin and the phenomenon of fibrinolysis. In our work washings from previous clots were always added to insure the presence of sufficient thrombin, and the fibrin was removed from the blood as soon as whipping was completed. With these precautions the method seemed reliable enough for the comparisons we have made. It was impossible to use the pre-

¹¹ LANGSTEIN und MAYER: Beiträge zur chemischen Physiologie, 1904, v, p. 69.

¹² WHIPPLE and HURWITZ: *Loc. cit.*

precipitation methods entirely on account of the large amount of blood required. However in every set of experiments a few determinations were made by the method of Reye. This method is more accurate than the former, but with the small amounts of blood at our disposal the error must have been correspondingly large. The percentage increase or decrease, however, always agreed closely with the first method. Determinations were not made by heat coagulation because the experience of most physiological chemists has been that such methods are unreliable. The coagulation point of a protein varies widely with the slightest differences in acidity, salt content, rate of heating, and even protein concentration. These factors are of course beyond control. Chick and Martin,¹³ after recently investigating anew the subject of heat coagulation of proteins, have even said that it is improper to speak of proteins as having any particular coagulation temperature at which they are thrown out of solution. In all cases the fibrin obtained by whipping or the fibrinogen precipitated by ammonium sulphate was dried for two hours at 100° C., replaced in the weighing bottle and kept in a desiccator over sulphuric acid. Weighings were then made daily until a constant weight within .2 mg. was obtained for three successive days.

RATE OF FIBRINOGEN REGENERATION.

That fibrinogen can be reformed in the body after its removal from the blood has been known many years. Dastre¹⁴ reported Magendie to have first discovered that the fibrinogen might be removed from an animal by successive bleedings, defibrination, and reinjection. In the horse at the end of fifteen to sixteen days the total amount of fibrinogen was greatly increased by this treatment. Dastre confirmed these results. Doyon¹⁵ has described experiments in one of which the amount of fibrinogen rose from .11 gm. to 1.8 gm. per 1000 c.c. of blood in nine and one half hours. Mathews found in the cat that in thirty-six to seventy-two hours the amount had returned to normal and afterwards might be found in excess. Whipple and Hurwitz also found a regenera-

¹³ CHICK and MARTIN: *Journal of physiology*, 1910, xl, p. 404.

¹⁴ DASTRE: *Archives de physiologie*, 1893, v, p. 169.

¹⁵ DOYON: *Comptes rendus de la Société de Biologie*, 1906, lix, p. 860.

tion in excess as the liver recovered from the effects of chloroform poisoning.

The first point to be determined in our experiments was whether this regeneration would occur under ether anaesthesia and in the four or five hours at our disposal for a single experiment. Accordingly a dog was placed under ether anaesthesia and samples of blood drawn from the carotid, defibrinated by whipping and reinjected in the femoral vein. In this manner about one fifth to one sixth of the total blood can be drawn at each bleeding. Eight or nine bleedings greatly lower the fibrinogen content of the blood, although it is difficult to render an animal fibrinogen free. During injection the blood was kept at body temperature. The amounts of fibrinogen as fibrin were determined at the beginning, after the last bleeding and at the end of the experiment. A protocol of one experiment is submitted.

Dog No. 6. March 31. — 10.15 A. M. Ether anaesthesia. Cannulas placed in carotid for bleeding and in femoral for reinjection.

10.31 A. M. Bled 51 c.c. Whipped until no more fibrin formed. Fibrin subsequently found to be .0802 gm. or 1.570 gm. per 1000 c.c. of blood.

10.38 A. M. Bled 97 c.c. and at same time reinjected previous sample. This order of bleeding, defibrination, and reinjection followed in all of the succeeding samples.

10.43 A. M. Bled 93 c.c.

10.47 A. M. Bled 66 c.c.

10.52 A. M. Bled 108 c.c.

10.58 A. M. Bled 95 c.c.

11.04 A. M. Bled 104 c.c.

11.08 A. M. Bled 101 c.c.

11.13 A. M. Bled 101 c.c.

11.18 A. M. Bled 110 c.c. This sample contained .019 gm. of fibrin or .172 gm. per 1000 c.c. of blood.

3.20 P. M. Bled 75 c.c. This sample contained .0264 gm. of fibrin or .352 gm. per 1000 c.c. of blood.

This experiment showed quite clearly that fibrinogen is regenerated even more rapidly than generally supposed. The rate of regeneration was determined in two other dogs. Table I summarizes these results. Under experimental conditions in from three to five hours after defibrination the fibrinogen may increase over 100 per cent.

REGENERATION OF FIBRINOGEN AFTER AN ECK FISTULA AND
LIGATION OF THE PORTAL VEIN.

So far as we have been able to find, no one has ever studied the fibrinogen content of the blood before and after an Eck fistula. Nolf and Doyon entirely extirpated the liver, although the literature occasionally credits them with having made a fistula. Our idea was to reduce the fibrinogen of the blood by whipping, make a fistula, and

TABLE I.

RATE OF FIBRINOGEN REGENERATION AFTER PARTIAL DEFIBRINATION. THE FIGURES
INDICATE THE AMOUNT OF FIBRINOGEN PER 1000 C.C. OF BLOOD.

Initial amount.	Whipped to.	Hours later.	Final amount.	Per cent increase
1.572	.172	4.	.352	104
2.106	.337	4.5	.525	55
1.756	.246	3	.523	112

then determine the rate at which it reappeared. If the liver normally built up the substance from some constituent of the portal blood, after the fistula and ligation of the portal close to the liver regeneration would be impossible.

In practice we found it necessary, in order to prevent hemorrhage, to do the fistula first and then defibrinate. In every case the veins were examined post mortem to make sure that the opening was patent and large enough to function. Washings from the previous clots were always added during the whipping to insure an excess of thrombin. A protocol of one of these experiments is given.

Dog No. 17. June 2.—9 A. M. Ether anæsthesia. Tracheotomy. Eck fistula completed at 9.50 A. M.

9.50 A. M. Bled 32 c.c. Fibrin found to equal 2.187 gm. per 1000 c.c. of blood.

10.08 A. M. Portal ligated close to the liver.

10.10 A. M. Bled 90 c.c. Whipped and reinjected.

10.15 A. M. Bled 50 c.c. Whipped and reinjected.

10.17 A. M. Bled 90 c.c. Whipped and reinjected.

Relation of the Liver to the Fibrinogen Content of Blood. 169

- 10.20 A. M. Bled 88 c.c. Whipped and reinjected.
10.30 A. M. Bled 90 c.c. Whipped and reinjected.
10.36 A. M. Bled 42 c.c. Fibrin in this sample equalled .414 gm.
per 1000 c.c. of blood.
10.45 A. M. 28 c.c. of blood drawn into 14 c.c. of sodium oxalate.
Fibrinogen by method of Reye equalled .720 gm. per 1000 c.c. of
plasma.
1.45 P. M. 28 c.c. of blood drawn and determined by method of
Reye. Fibrinogen equalled .900 gm. per 1000 c.c. of plasma.
1.46 P. M. Bled 50 c.c. and whipped. Fibrin equalled .512 gm.
per 1000 c.c. of blood.

This experiment showed a marked increase in fibrinogen after establishing the Eck fistula and ligating the portal. The result was hardly to be expected, judging from the work that has accumulated to show that the liver is the seat of fibrinogen formation. The protocol shows that the two methods of determination agree in the main. The figures for fibrinogen are naturally higher than for fibrin, since they refer to the plasma. The percentage increase, however, is close, being 23 per cent in one and 25 per cent in the other.

Table II gives the results of eight experiments similar to the one described above. In six of these there is a decided increase in the amount of fibrinogen. In the fourth there is a loss of 6 mg. per 1000 c.c. of blood, a figure of course within the limits of experimental error. The fifth experiment shows an appreciable loss of fibrinogen after the fistula. It may well be that in certain animals the shock of such an operation as the fistula has lowered the power of the fibrinogen forming parts. The data presented seem sufficient, however, for concluding that fibrinogen may be reformed even after the portal blood is entirely diverted from the liver.

REGENERATION OF FIBRINOGEN AFTER ECK FISTULÆ AND LIGATION
OF THE HEPATIC ARTERY.

Our hypothesis thus far had been that fibrinogen was made by the liver from constituents of the portal blood. The data presented in Table II show that either other organs may produce the substance or that the liver may use arterial blood for that purpose. To help settle this point we did the usual Eck fistula and in addition ligated the hepatic

artery. This amounted to an extirpation of the liver, since the only connection remaining with the circulation was the hepatic veins. A

TABLE II.

RATE OF FIBRINOGEN REGENERATION AFTER ECK FISTULE AND LIGATION OF THE PORTAL VEIN. FIGURES GIVE FIBRIN CONTENT OF 1000 C.C. OF BLOOD.

Initial amount.	Whipped to.	Hours later.	Final amount.	Per cent increase.
2.723	.264	4.5	.375	42
1.279	.358	3	.483	34
0.689	.093	2	.152	62
2.498	.348	1.75	.342	Loss
....	.906	2.5	.753	16 Loss
....	.345	2.75	.409	18
1.666	.416	3	.645	55
2.187	.414	3	.512	23

back flow into the liver through these channels has been discussed by Eck fistula workers.¹⁶ No doubt some blood might penetrate the liver in this way, but it is not generally believed that such a retrograde

TABLE III.

FIBRINOGEN CONTENT OF THE BLOOD AFTER ECK FISTULE AND LIGATION OF PORTAL VEIN AND HEPATIC ARTERY. FIGURES GIVE FIBRIN CONTENT OF 1000 C.C. OF BLOOD.

Initial amount.	Whipped to.	Hours later.	Final amount.
1.697	.174	2	.028
1.543	.254	1.5	.071
1.215	.167	2	{ Non-coagulable.

circulation could be of any importance. Table III summarizes three experiments carried out as just described.

¹⁶ HAHN, MASSEN, NENCKI, and PAWLOW: *Archiv für experimentelle Pathologie und Pharmacologie*, 1893, xxxii, p. 161.

Not only had no fibrinogen been formed in these experiments, but the amount remaining after partial defibrination had disappeared in large part or entirely. In the first two experiments the fibrinogen was still measurable, although there was not enough to form a normal blood clot. In the third no fibrin could be detected and the blood remained non-coagulable indefinitely. In all cases washings from normal clots as well as tissue extracts were added without results. There was evidently a reduction of coagulating proteid and in the last experiment an absence of the substance altogether.

Our experiments have shown that without any disturbance of the liver circulation fibrinogen may be regenerated as much as 100 per cent in three hours. With the portal blood diverted this rate of regeneration has dropped to an average of about 40 per cent. When the liver has been extirpated the power of regeneration has been entirely lost and the fibrinogen remaining in the blood has disappeared. The simplest explanation of this series of results is that the liver forms fibrinogen from some constituent of the blood. The richest blood supply to the liver is of course the portal, and from this source most of the fibrinogen is made. When this supply is removed, the liver makes use of the arterial blood and still continues to form a certain amount of fibrinogen. This explains why in all the Eck fistula literature there is no mention of the dogs' blood ever becoming non-coagulable, a fact that has been puzzling to those who believed in liver formation of fibrinogen. Anatomically there is no reason why arterial blood to the liver may not be used in this way. Confirmatory evidence that the liver may use arterial blood in its various activities has been offered by Voegtlin and Bernheim.¹⁷ They recently found that the formation of bile continues after an Eck fistula and ligation of the portal.

The only other explanation of our results seems to be that the liver may be sending a hormone to some distant organ, thus stimulating it to fibrinogen formation. This does not seem probable. If such a substance were constantly being thrown into the blood, one might expect enough of it to be present to keep up the fibrinogen formation for some time after extirpation of the liver. Fibrinogen, however, seems to decrease in amount at once.

In view of the work reported here it would seem probable that

¹⁷ VOEGLIN and BERNHEIM: *Journal of pharmacology and experimental therapeutics*, 1911, ii, p. 455.

the results obtained by Mathews on extirpating the intestines should be referred to the liver instead of the intestines. Our experiments showing that after removal of the liver there is a disappearance of fibrinogen confirm the work of Nolf. This author found, however, that it disappeared only after very special procedures, among which were injection of peptone and stasis in the intestinal area. Our experiments indicate that partial defibrination is another procedure hastening its disappearance.

TABLE IV.

DECREASE IN FIBRINOGEN AFTER ECK FISTULE AND LIGATION OF BOTH PORTAL VEIN AND HEPATIC ARTERY. FIGURES GIVE THE FIBRIN CONTENT OF 1000 C.C. OF BLOOD.

Initial amount.	Hours later.	Final amount.
3.766	1.5	3.503
2.217	2.5	1.934
Same dog	3.5	1.728
2.612	4	2.462
1.118	4	.955

The removal of the fibrinogen in this last condition might best be explained by assuming that the body normally uses the substance in some way. In our work we had merely whipped out a large part of the fibrinogen and the remainder was quickly used up. To get some basis for this idea four experiments were run to find at what rate fibrinogen was taken from the blood when its source of supply was cut off. An Eck fistula was made and both portal vein and hepatic artery tied without any defibrination. The initial amount of fibrinogen was determined and other estimations made after a period of a few hours.

In Table IV the results of four such experiments are summarized. These experiments all agree in showing a reduction in the amount of fibrinogen after the isolation of the liver. The decrease, however, is not very marked. It is about the same in each case, and it seems to have no relation to the time. These considerations have led us to doubt whether we have any evidence of fibrinogen consumption in such

regular decreases. The diminution at the end of two or three hours is probably no greater than we should expect from the dilution of the blood occasioned by the absorption of water from the tissues in order to make up for the volume lost by the hemorrhage of the operation and the withdrawal of blood for the analysis. The disappearance of fibrinogen in chloroform poisoning, as reported by the several workers, seems to be sufficient evidence, however, for believing that fibrinogen is utilized in some way by the body. Quite likely our experiments did not allow sufficient time for the reduction in amount to become clearly apparent.

SUMMARY.

1. Fibrinogen is regenerated at a rapid rate in the dog after partial defibrination. In three hours the amount may be increased as much as 100 per cent.
2. After an Eck fistula and ligation of the portal vein, fibrinogen is still reformed after partial defibrination but at a slower rate.
3. After an Eck fistula, ligation of both portal vein and hepatic artery and partial defibrination, fibrinogen is no longer reformed, and the amount remaining in the blood rapidly disappears.
4. The experiments reported do not clearly show a reduction in fibrinogen after extirpation of the liver without partial defibrination.
5. The simplest explanation of these facts is that the liver itself forms fibrinogen. The necessary material is furnished by the blood of the portal vein for the most part, but if this supply is cut off the liver makes use of material reaching it by way of the hepatic artery. The disappearance of fibrinogen from the blood after its seat of formation is removed probably means that the substance plays some rôle in bodily metabolism. Another explanation of the phenomena observed might be that the liver conditions the formation of fibrinogen in some distant organ by means of a hormone or other stimulating mechanism.

FASTING STUDIES.—IX. ON THE DIFFERENTIAL LEUCOCYTE COUNT DURING PROLONGED FASTING.¹

BY PAUL E. HOWE AND P. B. HAWK.

[From the Laboratory of Physiological Chemistry of the University of Illinois.]

CHANGES in the distribution of the various forms of leucocytes in the blood during prolonged fasting have received but little attention. In the majority of the fasts reported, the observations were confined to the enumeration of the erythrocytes and leucocytes and the estimation of the hæmoglobin.

Tauzk² examined the blood of Succi, and observed a decrease in the relative number of mononuclear lymphocytes and an increase in the eosinophile and polymorphonuclear leucocytes during a thirty-day fast.

Benedict,³ in a more recent study of the variations in the different forms of leucocytes as the result of fasting, reports results from the blood of one individual during two fasts, accompanied by data from the intervening and final feeding periods. The subject presented, normally, a condition of leucocytosis. The differential count of the leucocytes showed in general an increase in the number of polymorphonuclear leucocytes in both fasts; he states, however, that "in neither instance did the percentage reach a distinctly pathological level." The small lymphocytes were below the normal during both fasts, with two exceptions, the second and final days of the seven-day fast. The relative number of large lymphocytes was above normal during the last two days of the seven-day fast and entirely above during the following fast.

¹ Reported before the American Society of Biological Chemists at the New Haven meeting, December, 1910. Proceedings of the Society of Biological Chemists, 1910, p. 15.

² TAUZK: Orvosi hetilap, Budapest, 1894, 512; abstract from Jahresbericht über Thier-Chemie, 1894, xxiv, p. 147.

³ BENEDICT: Metabolism in inanition, Carnegie Publication, 1907, No. 77.

The eosinophiles were in general low and the mast cells high during both fasts.

Charteris⁴ reports the percentage distribution of the leucocytes of Beauté during a fourteen-day fast, and calls especial attention to the gradual increase in the number of eosinophilic leucocytes. He does not lay any stress upon the variations in the other forms of leucocytes, and a consideration of the data presented shows that while there appears to be a tendency for the polymorphonuclear leucocytes to increase and for the small lymphocytes to decrease, still the variations which occur do not greatly exceed the possible normal fluctuation in the differential distribution of these cells.

The results of experiments reported upon dogs (Luibomudrow,⁵ Keuthe⁶) are conflicting. Luibomudrow investigated the changes in the various forms of leucocytes of fifteen dogs which fasted to a loss of 20 per cent of the original body weight. His data indicate an increase in the percentage of polymorphonuclear leucocytes, a decrease in the percentage of the small mononuclear lymphocytes and the presence of eosinophiles (which did not occur normally) as the results of fasting. Keuthe's data, on the other hand, indicate exactly the opposite effect; his observations were made upon but one dog during two short fasts, one of eight days and the other of three days, the preliminary periods of which were entirely different. This latter fact is of great significance, since he shows in the same paper that the diet exerts an influence upon the relative numbers of the various forms of leucocytes in the blood stream.

Poletaew,⁷ Okintschitz,⁸ and Kallmark⁹ have studied the changes in the leucocytes in the blood of rabbits as the result of fasting. These authors report a decrease in the relative number of both the polymorphonuclear leucocytes and of the lymphocytes. Poletaew and Okintschitz also demonstrate an increase in the number of large mono-

⁴ CHARTERIS: *Lancet*, 1907, p. 685.

⁵ LUIBOMUDROW: *Dissertation*, St. Petersburg, 1893, quoted by MUHLMANN: *Centralblatt für allgemeine Pathologie*, 1899, x, p. 183.

⁶ KEUTHE: *Deutsche medicinische Wochenschrift*, 1907, xxxiii, p. 588.

⁷ POLETAEW: *Archives de sciences biologiques de St. Petersburg*, 1893, ii, p. 795.

⁸ OKINTSCHITZ: *Archiv für experimentelle Pathologie und Pharmakologie*, 1893, xxxi, p. 383.

⁹ KALLMARK: *Folia hæmatologica*, 1911, xi, pt. i, p. 411.

nuclear lymphocytes and of the eosinophiles. The data reported by Kallmark show an increase in the number of eosinophiles, but do not demonstrate the changes in the large mononuclear lymphocytes, since he includes this form of cell with the small lymphocytes. After the initial decrease in the number of lymphocytes Kallmark shows that the percentage of this form of cell remains practically constant at all times, there being a decrease at the beginning of the fast, which returns to approximately the normal and an increase upon the ingestion of food. His explanation of this phenomena will be considered later.

The differential count of the leucocytes of normal dogs has been made by Busch and Bergen,¹⁰ who show the following relations: Lymphocytes 21 per cent, large mononuclear lymphocytes 6.8 per cent, polymorphonuclear leucocytes 65.7 per cent, eosinophiles 5.3 per cent, and mast cells rare. They state that the polymorphonuclear leucocytes show the least, and the eosinophiles the greatest, variation; with a high polymorphonuclear percentage there is a small percentage of lymphocytes, and with a high percentage of eosinophile there is a low percentage of polymorphonuclear leucocytes.

DESCRIPTION.

Differential counts were made of the leucocytes on blood smears obtained during the fasts of four dogs and two men. The lengths of the fasts were as follows: Dog No. 1, two fasts — one of fifteen days and a second of thirty days; Dog No. 2, a fast of forty-eight days; Dog No. 3, a fast of one hundred and seventeen days,¹¹ and Dog No. 6 (a pup), a fast of seven days. The two men fasted simultaneously for seven days. The blood was examined during an intermediate feeding period of forty-eight days in the case of Dog No. 1 and during a period of eight days following the fast in the case of the men. An examination of the blood under normal conditions previous to the fast was made in each case. Each subject received a constant quantity of water per day. The conditions of experimentation and a description of the subjects are found in previous publications from this laboratory.¹¹ The blood utilized in the preparation of the smears

¹⁰ BUSCH and BERGEN: Report of the Laboratory of the University of Buffalo, 1903, No. 2 (*Folia hæmatologica*, 1904, i, p. 783).

¹¹ HOWE, MATTILL, and HAWK: Proceedings of the American Society of Bio-

was taken from the ear in the case of the dogs and from the finger in the case of the men. The staining was done by the method devised by MacNeal.¹² A distinction was made between the following types of leucocytes, polymorphonuclear leucocytes, small lymphocytes, large lymphocytes, transitionals, eosinophiles, and basophiles. The results contained in the accompanying tables show only the *changes* which occurred in the percentage distribution of the leucocytes; *i. e.*, in cases where there was a variation of more than 1 to 2 per cent, this result is reported and all subsequent counts are at this value until another change occurs. Where individual days are recorded these are counts for but the one day. The data recorded as normal are an average of at least two counts. Wherever a marked change in the percentage distribution occurred a count was made upon a second smear and the average taken.

DISCUSSION.

The distribution of the differential forms of leucocytes in the blood of dogs under normal conditions shows but slight variation. This may be seen from the accompanying illustrative tables. An average of the results obtained, including the two normal periods of Dog No. 1, is as follows: polymorphonuclear leucocytes 48 per cent, small lymphocytes 45 per cent, large lymphocytes 4 per cent, transitionals 1.3 per cent, eosinophiles 1.1 per cent, and basophilic cells occasional. These results differ from those of Busch and Bergen,¹³ being lower in the percentage of polymorphonuclear leucocytes, large lymphocytes, and eosinophiles, and higher in the percentage of small lymphocytes. This difference may be due to the dietary variation, which may exert an influence upon the percentage distribution of the leucocytes (Keuthe).¹⁴

As the result of an initial fast three of the dogs (1, 3, and 6) showed

logical Chemists, 1909, p. 260; Journal of the American Chemical Society, 1911, xxxiii, p. 568; Journal of biological chemistry, 1911, x, p. 417, and 1912, xi, p. 103; Howe and Hawk: Journal of the American Chemical Society, 1911, xxxiii, p. 215.

¹² MACNEAL: Journal of the American Medical Association, 1907, xlviii, p. 609.

¹³ BUSH and BERGEN: *Loc. cit.*

¹⁴ KEUTHE: *Loc. cit.*

TABLE I.
DIFFERENTIAL LEUCOCYTE COUNT. (PERCENTAGES.)

DOG NO. 3. NORMAL AND FASTING.						
Day.	Poly.	Small.	Large.	Trans.	Eosin.	Baso.
Normal . . .	52.8	41.4	2.8	1.6	0.8	0.6
1	68.0	28.4	2.0	1.6	0.4	1.2
10-26	41.8	53.6	3.2	0.4	0.0	1.0
54	26.0	69.2	2.8	2.0	0.0	0.0
59	28.8	65.2	4.0	1.2	0.8	0.0
(H ₂ O) 62	28.4	63.2	7.6	0.0	0.8	0.0
75-82	46.0	48.4	4.4	1.2	0.0	0.0
83-100	34.8	60.0	3.2	2.0	0.0	0.0
106	18.4	76.0	2.0	3.2	0.4	0.0
112	24.8	67.2	6.4	1.6	0.0	0.0
FEEDING.						
3	34.8	54.0	10.4	1.2	0.0	0.0
DOG NO. 1. NORMAL AND FIRST FAST.						
Normal . . .	49.2	43.6	4.8	0.8	1.2	0.4
1	36.0	59.2	2.8	2.0	0.0	0.0
4	37.6	57.6	3.6	0.8	0.0	0.4
7	40.8	54.8	3.2	0.8	0.4	0.0
11	26.4	69.6	3.2	0.8	0.0	0.0
FEEDING.						
1	48.0	50.8	1.2	0.0	0.0	0.0
47	47.2	45.2	4.4	2.0	0.8	0.4
SECOND FAST.						
1-13	46.4	49.6	2.4	1.2	0.0	0.4
20-29	44.4	52.0	3.2	0.4	0.0	0.0

a decrease in the percentage of polymorphonuclear leucocytes, with an accompanying increase in the percentage of small lymphocytes, while the blood of the remaining dog (2) showed exactly the reverse

TABLE II.
DIFFERENTIAL LEUCOCYTE COUNT. (PERCENTAGES.)

SUBJECT "E."						
Day.	Poly.	Small.	Large.	Trans.	Eosin.	Baso.
Normal . . .	61.2	32.2	4.4	1.2	1.0	0.0
FASTING.						
1	66.0	26.8	6.0	0.8	0.4	0.0
2	58.8	37.2	2.8	0.8	0.4	0.0
4	64.0	28.4	4.0	1.6	2.0	0.0
6	62.0	32.0	2.0	0.0	4.0	0.0
7	58.8	33.2	2.8	0.8	4.4	0.0
FEEDING.						
1	68.4	26.0	1.6	0.4	3.6	0.0
3	52.8	40.8	3.2	0.4	2.8	0.0
6	61.6	34.0	3.2	0.0	1.2	0.0

condition. The changes in the number of large lymphocytes were variable; they increased gradually in the case of Dogs 3 and 6 and decreased and remained fairly constant in the case of Dogs 1 and 2. The variations in the transitional, eosinophilic, and basophilic leucocytes are too small to be of special significance except in the case of Dog 2. The smears from this animal showed a distinct increase in the number of eosinophiles (0.4 to 7.6 per cent) at the beginning of the fast, followed by a progressive decrease until the fast terminated (7.6 to 0.0). This latter finding was probably due to the physical

condition of this dog, which was distinctly emaciated and anemic at the beginning of the fast.

The repeated fast of Dog No. 1 did not show the same changes in the differential distribution of the leucocytes as occurred in the initial fast. During the repeated fast all of the forms of cells with the exception of the large lymphocytes remained practically constant, although the fast was twice the length of the first fast (fifteen days and thirty days). This constancy observed in the differential counts of the second fast we would interpret as due to the influence of repeated fasting. Upon the ingestion of food following the first fast the leucocytes returned to their normal relative proportions.

The decrease in the percentage of polymorphonuclear leucocytes and the increase in the small lymphocytes in the case of the normal dogs as the result of a prolonged fast were of practically the same magnitude. The value at the end of each fast was between 26.4 per cent and 21.2 per cent for the polymorphonuclear leucocytes, or a decrease of approximately 50 per cent from the normal, and between 69.6 per cent and 63.2 per cent for the small lymphocytes, or an increase of about 50 per cent as compared with the normal distribution. These changes, which occurred as the result of prolonged fasting (until the inception of the premortal rise in nitrogen excretion), are more nearly in accord with those obtained by Liubomudrow¹⁵ in his shorter fasts. The constant relation observed by Kallmark¹⁶ between the leucocytes in the case of rabbits is not apparent in our data. This author considers that the thymus and lymph glands are concerned with the maintenance of a constant relation between the various forms of cells. The variations observed as the result of fasting and of the ingestion of food after a fast he ascribes to a lag in the activities of these organs such that with the increased consumption of lymphocytes, which he conceives as taking place, there is not an immediate increased production of lymphocytes; the result is a temporary decrease in this form of cell.

The data obtained from the two fasting men indicate an increase in the percentage of the polymorphonuclear leucocytes at the beginning of the fast, followed by a decrease below the normal at the end of seven

¹⁵ LIUBOMUDROW: *Loc. cit.*

¹⁶ KALLMARK: *Loc. cit.*

days; the opposite conditions held for the lymphocytes. There was an increase in the percentage of large lymphocytes during the earlier part of each fast. Subject E showed an increase in the eosinophilic leucocytes which continued through the post-fasting feeding period.

As the result of the ingestion of food following the fast there was a tendency for all the forms of leucocytes to return to the normal.

ON THE RELATION OF VENTRICULAR TONUS TO THE CAUSATION OF THE HEART BEAT.

By E. G. MARTIN.

[From the Physiological Laboratory in the Harvard Medical School.]

THE problem of the causation of the heart beat has been studied by many investigators and from various angles. One fruitful line of research has concerned itself with the effects of solutions of the blood salts on rhythmic activity. In experiments of this sort the tissues under examination often show, in addition to specific rhythmicity, well-marked tonus influences. These latter have seemed, in the experience of certain investigators, to be so constantly associated with particular conditions of rhythmicity as to indicate a fundamental relationship between the influence of the blood salts on cardiac rhythmicity on the one hand and on cardiac tonus on the other. Thus Howell,¹ in analyzing the heart's automatic activity, associates the functions of sodium and calcium salts in the rhythmic process with definite tonus influences exerted by them. His position, in brief, is that the tone loss shown by heart tissue in solutions of sodium salts indicates that sodium is concerned in the relaxation process; while the increased tone produced by calcium shows calcium to be concerned in the process of contraction.

S. R. Benedict² has advanced a more elaborate hypothesis in which he assumes that the ability of heart muscle to show spontaneous activity is definitely associated with a certain tonic state. The effects of various substances upon cardiac rhythmicity depend, according to Benedict, on whether they raise tone or lower it.

The question seems to me of enough importance to merit a more exhaustive consideration than it has yet had. I have therefore made a detailed study of the tone changes shown by ventricular tissue of

¹ HOWELL: this Journal, 1901, vi, p. 199.

² BENEDICT: this Journal, 1905, xiii, p. 192; also 1908, xxii, p. 16.

turtles in more than one hundred and fifty experiments, extending over a period of eight years, and including a large variety of experimental conditions.

Method of comparing tone changes.—In most of my experiments with ventricular tissue the strip method of Greene³ has been used. This method is highly satisfactory in general. When one attempts, however, to make comparative studies of the tone changes shown by a large series of strips, the difficulty of establishing a valid criterion for the extent of the changes is at once encountered. To measure tone variations in percentages of resting length is objectionable because the resting length itself changes with every change in tone. Moreover, in cutting a succession of strips there is every likelihood that the arrangement of contractile elements will vary greatly from strip to strip, so that the measured length and the effective length may easily be quite different.

As a basis for comparative study of tone changes I have selected the maximal height of spontaneous contraction shown by the strip when immersed in 0.7 per cent sodium chloride. This value is one which occurs in nearly all experiments with ventricular strips, since the usual method of initiating spontaneous beats in such strips is by immersing them in sodium chloride solution. The height of contraction can be measured with great accuracy on the same record on which tone changes are measured. The magnification of the heart lever need not be taken into account, since it is the same, in any experiment, both for contraction height and for tone variations.

These are practical reasons why the recorded height of contraction is a useful basis on which to compute percentage tone changes. Its employment involves the assumptions that the height of contraction of heart strips subjected to simple and uniform treatment will vary with the effective length, and that the effective length for ordinary contractions bears a reasonably constant relation to the effective length for tonus variations. Our knowledge of the actual mechanism of ordinary and tonus contractions is not adequate either to prove or disprove these assumptions, and in the absence of knowledge assumptions must be used with caution. I consider them justified in this instance by the experimental observation that in strips immersed in

³ GREENE: this Journal, 1898, ii, p. 82.

sodium chloride solution the tone loss bears a definite relation to the initial height of contraction whether this height be great or small. I have studied records of thirty-two experiments in which strips were immersed in 0.7 per cent sodium chloride solution to the point of "exhaustion." The initial height of contraction, as shown on the record, ranged from 13 mm. to 30 mm. I distributed the experiments in five groups according to their initial contraction heights and obtained the following average hour percentage tone losses.⁴ For a contraction height of 13-15 mm. (three experiments) the average was 26 per cent; 16-18 mm. (five experiments), 30 per cent; 19-21 mm. (eight experiments), 26 per cent; 22-24 mm. (ten experiments), 25 per cent; 25-30 mm. (six experiments), 24 per cent. The hour percentage tone loss held practically constant, while the height of contraction more than doubled—a result that could scarcely be purely accidental, and that indicates a definite relationship between initial height of contraction and tonus loss, on the one hand, and effective length on the other.

Media employed.—In choosing the various media whose effect upon ventricular tissue was to be studied, I have been guided throughout my work by two principles: first, that the only substances to which we know the heart to be adapted are those present in the blood; as soon, therefore, as we subject it to the influence of other substances than those, we introduce abnormal conditions, and thereby lessen the value of whatever conclusions we may wish to draw from our observations; second, that the heart is adapted to the blood constituents in certain definite, so-called physiological, concentrations; we are

⁴ Since different experiments occupy different amounts of time, allowance must be made for the duration of the tonus effect. This allowance I have made by reducing the observations to an hour basis. Comparisons are thus furnished between hour percentage changes. For example, if a strip gives on the record an initial height of contraction of 20 mm. and in a period of two hours relaxes so as to show a fall of base line of 8 mm., the actual hour tone loss is 4 mm. and the hour percentage loss 20 per cent. Where the period of the tone change under observation was less than an hour I treated it as though the change would continue uniformly till the hour ended, multiplying the observed tone change by the fraction of an hour it endured. Tone changes continuing for longer periods than an hour were treated as though they were uniform throughout their period, unless there were obvious variations in the rate of change; in such cases the period was subdivided so as to give substantially uniform rates of change for each interval.

not entitled to interpret effects produced by solutions excessively concentrated as nothing more than higher degrees of the effects produced by the same solutions in physiological concentrations; there may be qualitative as well as quantitative differences. This possibility, which has recently been pointed out by Hoskins⁵ for adrenalin, applies with equal force to all substances by which tissues are affected.

The chief media I have used are 0.7 per cent sodium chloride, Ringer's solution (0.7 per cent sodium chloride, 0.026 per cent calcium chloride, 0.04 per cent potassium chloride), a mixture of sodium and calcium chlorides in the same proportion as in Ringer's solution, 0.025 per cent calcium chloride solution, and 0.7 per cent sodium chloride made alkaline with sodium carbonate. Some or all of these media have been used, freed as far as possible of oxygen by brief boiling, saturated with atmospheric air or with pure oxygen, or in some cases having bubbling through them carbon dioxide, hydrogen, or nitrogen. Solutions through which hydrogen is bubbling are of course abnormal to heart tissue. My use of this gas was to minimize the taking up of atmospheric oxygen by the solutions at the surfaces. There was never any indication that the gas exerted positive influence on the tissue. I have obtained very interesting results by withdrawing strips from the solutions in which they were bathed and leaving them in moist air. This treatment seems to me to be valid, in view of the complete saturation of the tissue with the solution from which it is taken. The effect is to confine the interchanges between cells and bathing medium to the restricted amount of the medium contained in the intercellular spaces. Since there is thus likely to be a somewhat prompt establishment of equilibrium throughout the mass, the experiments with moist air may be peculiarly instructive.

The effect on tone of isolating ventricular tissue from its blood supply.—The mechanical stimulation inevitable in the preparation of ventricle strips for experimentation throws them into a state of strong tonic contraction. This tonus of manipulation resulting from excessive mechanical stimulation possibly corresponds to the tonus of excessive electrical stimulation noted by Fischel⁶ and others. The first effect observed after the strip has been placed in position is

⁵ HOSKINS: this Journal, 1912, xxix, p. 366.

⁶ FISCHEL: Archiv für experimentelle Pathologie und Pharmakologie, 1897, xxxviii, p. 228; SCHULTZ: this Journal, 1908, xxii, p. 136.

a rather rapid relaxation from the condition of high manipulative tone. This first relaxation seems to be essential. I know of no bathing medium which hinders it appreciably. The rapid initial relaxation continues for not more than twenty or twenty-five minutes at the longest. Some average hour percentage values for the initial relaxation, compared with the heights of subsequent contractions in sodium chloride solution, were: for 0.7 per cent sodium chloride, 250 per cent; for 0.7 per cent sodium chloride saturated with oxygen, 200 per cent; for sodium chloride and calcium chloride in the proportions used in Ringer's solution, 230 per cent; for moist air, 350 per cent.

Following the initial rapid relaxation, the characteristic tone change of excised ventricle tissue is a much slower but continued relaxation which may go on for many hours. I have numerous records of continuous relaxation during twelve to fifteen hours, and several where the strip relaxed steadily for twenty-four hours. The rate of this secondary relaxation is higher during the first hour or two than later, and is modified to some extent by the medium surrounding the tissue. Thus the average hour percentage loss during the early part of the period of secondary relaxation was for 32 strips in 0.7 per cent sodium chloride, 26 per cent; for 4 strips in Ringer's solution, 40 per cent; for 7 strips in moist air, slightly above 50 per cent; for 5 strips in sodium chloride saturated with oxygen, 26 per cent; for 5 strips in sodium and calcium chlorides in the proportion of Ringer's solution, 24 per cent. All these figures are concerned with a period of secondary relaxation lasting about one hour. The media in which relaxation continued for a long time were sodium chloride, kept saturated with oxygen, and moist air applied after sodium chloride exhaustion. The average duration of activity of strips in sodium chloride kept saturated with oxygen was about ten hours; the average hour percentage loss for the entire period was 7 per cent. Strips which have been "exhausted" in sodium chloride solution and then transferred to moist air virtually always, in my experience, continue to relax in this latter medium, often for many hours. In twelve experiments the average duration of the moist-air period was thirteen hours, the average hour percentage tone loss was 4.5 per cent.

Media which induce increased tonus.—Benedict⁷ lists as the strongest

⁷ BENEDICT: *Loc. cit.*, p. 28.

tone-raisers, oxygen, calcium chloride, and sodium carbonate, and attributes the improvement in beat induced by these substances after sodium chloride exhaustion to their tone-increasing influence. I have observed the effects of these substances in a great many experiments, and have been led by my observations to differ somewhat from Benedict's statements, both with reference to the tone-raising properties of these substances and to the relationship between their tonus influence and their effects upon rhythmicity. One of Benedict's strongest tonus increasers is oxygen. I have pointed out elsewhere⁸ that the rhythmic activities of heart strips are promoted by abundant supplies of atmospheric oxygen as well as by treatment with pure oxygen. Two situations in which improved beat follows oxygen application are: passing a stream of air or oxygen through a 0.7 per cent sodium chloride solution in which a strip is nearly or quite exhausted; and transferring a strip exhausted in sodium chloride solution to moist air. In eight experiments in which air or oxygen was turned into a sodium chloride solution with consequent improvement of beat of the contained strip, the tone continued to fall in seven experiments, but rose for an hour in one, later falling again for five hours. In twenty-nine experiments in which recovery from sodium chloride exhaustion followed transfer of the strip to moist air, relaxation continued uninterrupted in twenty-eight, and in one there was evidence of a slight tone increase at the moment of transfer, followed shortly by continuous relaxation. In some of these experiments the moist chamber was filled with pure oxygen, without obvious difference of effect on the tissue.

While I differ thus from Benedict with regard to the tone-raising powers of oxygen, I wish to recall what I have previously reported,⁹ that strips subjected to the combined influence of oxygen and calcium chloride show much greater tonus increases than when treated with either substance by itself. My contention is not that oxygen is never under any circumstances a tone-raiser, but that its influence on tone is distinct from its power to bring about increased activity.

I have not observed the effects of immersing strips in pure sodium carbonate solutions, but excellent recovery after sodium chloride exhaustion can usually be obtained by the use of sodium chloride

⁸ MARTIN: this Journal, 1906, xv, p. 307.

⁹ MARTIN: *Loc cit.*, pp. 308, 311.

solution made alkaline with sodium carbonate. This alkaline solution, in my experience, is not a constant tone-raiser, nor is recovery of function following its use always associated with rising tone. I have many records of excellent recovery with continuous loss of tone, and a number in which the alkali induced a marked rise in tone without causing any improvement in beat.

The blood constituent which most consistently brings about a rise in tone in heart strips is calcium chloride. Even this substance, however, by no means invariably increases cardiac tone. I have pointed out above (page 186) that fresh strips bathed in mixtures of sodium and calcium chlorides in the same proportion as in Ringer's solution show continuous relaxation for considerable periods. In order to obtain tone increase in fresh strips by means of calcium chloride, the solution must be oxygenated or else the concentration of the salt must be markedly greater than in Ringer's solution.

Calcium chloride in physiological concentration acts most effectively to cause increase in tone when applied to strips that have been previously exhausted in sodium chloride solution. I have pointed out elsewhere¹⁰ that in this situation also the reviving power of calcium chloride is most marked. Are we to conclude from this coincidence that the power of calcium chloride to bring about recovery is necessarily associated with its tone-raising property? A careful analysis of the behavior of strips under treatment with calcium chloride shows that the two properties may well be wholly independent. In ten experiments in which excellent recovery of inactive strips was brought about by calcium chloride I determined the time during which the tone rose, and the time elapsing between the point of maximum tone and the point where the tone fell again to the level it showed before the calcium chloride was applied. The period of rising tone varied between one and seven minutes, averaging 3.4 minutes. The time required for the tone to fall to the former base line varied between four and forty minutes, averaging 17.6 minutes. In every one of these experiments the improvement in beat persisted for hours after the application of the calcium-containing solution, and was accompanied by a continuous fall in tone. Obviously the activity of the tissue in these experiments did not depend on a state of increased tone, for the

¹⁰ MARTIN: this Journal, 1904, xl, pp. 119, 126.

improvement in beat persisted long after the tone had fallen below the point of the previous inactivity.

The facts I have reported in the preceding paragraphs show, I believe, that Benedict's contention that oxygen, sodium carbonate, and calcium chloride owe their favorable influence on cardiac activity to their tone-raising property is not supported by experimental evidence. Benedict's further contention¹¹ that "exhaustion" in heart tissue signifies too great loss of tone, fails likewise in the face of the numerous instances of pronounced recovery accompanied by still further loss of tone. Of these the most marked are cases in which strips are transferred from sodium chloride solution to moist air. I have shown in an earlier paragraph that relaxation continues after this treatment, and I have elsewhere¹² emphasized the striking recovery which it induces.

Effects of sugar solutions. — Howell¹³ and others report that under certain circumstances heart strips immersed in sugar solutions show marked improvement in beat. Benedict¹⁴ calls attention to the rise in tone which accompanies this improvement, citing it as a point in support of his contention that tone increase bears a causal relation in respect to heightened activity. While this observation as one of a series of corroborative data would be of value, by itself it has little weight, and what importance it might have is lessened on account of the highly abnormal character of the treatment involved.

Do sodium salts cause relaxation? — Howell has laid considerable stress on the tendency of sodium salts to cause relaxation in heart tissue,¹⁵ associating this tendency with the function of sodium in connection with the causation of the beat. My observation, recorded above (p. 186), that heart tissue cut off from its normal blood supply may relax steadily for hours under various treatments, has led me to question whether sodium salts are as definitely relaxing as they have been supposed to be. Careful analysis of a large series of records has brought out the following significant facts: 1. During a period of about two hours after isolation heart strips relax fully as fast when

¹¹ BENEDICT: this Journal, 1905, xiii, p. 199.

¹² MARTIN: *Loc. cit.*, p. 124.

¹³ HOWELL: *Loc. cit.*, p. 185.

¹⁴ BENEDICT: *Loc. cit.*

¹⁵ HOWELL: *Loc. cit.*, p. 199, and 1906, xv, p. 284.

transferred from the body directly to moist air as when immersed in 0.7 per cent sodium chloride. 2. In the later stages of long experiments, when heart strips have been active for hours in moist air or in Ringer's solution, transfer from these media to 0.7 per cent sodium chloride is more apt to be followed by a rise in tone or no change at all than by a loss of tone. In nineteen experiments of this sort transfer to sodium chloride was followed by rising tone in twelve, in four there was no tone change, and in only three was there a definite tone loss. In all these cases the strips in sodium chloride solution showed the activity characteristic for that treatment.¹⁶ 3. The relation of sodium chloride to tone change is the same in hearts perfused *in situ* as in excised strips. Howell¹⁷ describes the relaxation due to loss of tone in hearts perfused with 0.7 per cent sodium chloride, and elsewhere¹⁸ states his belief that this tone loss is a specific sodium chloride effect. I have tested this idea by comparing in turtles' hearts *in situ* the effect on tone of simply cutting off the flow of blood through the heart, with the effect of perfusing with sodium chloride solution. In comparing tone changes in an empty heart with tone changes in a perfused heart certain precautions must be taken to make the comparisons valid. In the first place, the base of the ventricle must be carefully immobilized so that true tone changes may be distinguished from adventitious movements. This immobilization I accomplished by thrusting a stout needle horizontally through the roots of the arteries, and clamping the needle firmly in position. In the second place, the perfusion must be carried on in a manner that will not permit mechanical distention to obscure true tone changes. For obtaining adequate perfusion without distention I placed the inflow cannula in one of the venæ cavæ, tying the others off, and secured a perfectly free outflow by cutting through the walls of the ventricle near the apex in two or more places. Records of the behavior of the heart were gotten by carrying a thread from the frenum over a pulley to a recording lever. The tension on the heart did not exceed 2 gm. The heart was prepared for perfusion in the manner described, and a record of its behavior taken during a period of two or three hours. Then sodium chloride solution was passed through it and the record continued. In drawing

¹⁶ GREENE: *Loc. cit.*, p. 91; also MARTIN: this Journal, 1904, xi, p. 125.

¹⁷ HOWELL: this Journal, 1906, xv, p. 284.

¹⁸ HOWELL: *Ibid.*, 1901, vi, p. 199.

conclusions as to tone changes the record of the first half-hour was disregarded, so that the effects of manipulation might have passed off. In three experiments of this sort the hour percentage tone losses before perfusion were, respectively, 23, 10, and 50 per cent, averaging 28 per cent; and after perfusion, respectively, 15, 18, and 28 per cent, averaging 20 per cent.

In several other experiments in which quantitative comparisons were not sought, direct observation showed that hearts cut off from the circulation relax to as great an extent as do hearts perfused with sodium chloride solution.

On the basis of the various observations cited above I am led to believe that the relaxation commonly noted in perfused hearts or in heart strips is a result of their separation from the circulation rather than a specific effect of sodium chloride.

SUMMARY.

1. Ventricular tissue, cut off from the circulation, relaxes steadily for several hours in moist air, in 0.7 per cent sodium chloride solution, in Ringer's solution, and in a mixture of sodium and calcium chlorides in the same proportion as in Ringer's solution. This relaxation is more rapid during the first two hours than later. It may continue for twenty-four hours or more.

2. The improvement in beat of ventricle strips following treatment with oxygen, sodium carbonate, or calcium chloride is not necessarily dependent on the tonus-increasing powers of these substances, since investigation reveals numerous instances in which the improvement in beat follows the use of these agents without corresponding tone increase, and others in which tone increase fails to be accompanied by improvement in beat.

3. Sodium chloride "exhaustion" cannot be explained as due to excessive loss of tone, inasmuch as excellent recovery can be had with still further loss of tone.

4. Evidence is presented indicating that the relaxation of heart tissue in sodium chloride solution is not so much the result of a specific action on the part of the solution as an effect of separation from the circulation.

THE RELATION OF THE ADRENAL GLANDS TO BLOOD PRESSURE.

BY R. G. HOSKINS AND C. W. McCLURE.

[From the Laboratory of Physiology of the Starling-Ohio Medical College.]

THAT adrenal extract exerts a powerful stimulating effect upon the sympathetic nervous system has been demonstrated by Elliott¹ and others. Recent researches at the Harvard Medical School have shown that during periods of particular stress the adrenal glands are stimulated to an augmented secretion which reinforces the sympathetic activity characteristic of such period. In cats, under the influence of anger or fear, partial asphyxia and strong sensory stimulation epinephrinemia has been demonstrated.²

Whether, however, during periods of quiet existence, the sympathetic nervous system by a minimal epinephrin discharge is kept in tonic activity is questionable. Such is the usual assumption, but it is without adequate foundation. It is based largely upon a supposition that minimal quantities of circulating epinephrin have an effect qualitatively similar to that of the relatively large quantities that have been used in experimental investigations. Such an assumption, however, is not necessarily true. It is possible that the threshold of stimulation of the sympathetic system is sufficiently high not to be reached by the normal concentration of circulating epinephrin. In the case of the small intestine a stimulating effect of adrenalin has been noted from quantities which left the sympathetic inhibitory fibres unaffected.³

Of the bodily functions controlled by the sympathetics blood pressure is one of the most sensitive to epinephrin. For this reason, and

¹ ELLIOTT: *Journal of physiology*, 1905, xxxii, p. 401.

² CANNON and DE LA PAZ: *this Journal*, 1911, xxviii, p. 64; CANNON and HOSKINS: *Ibid.*, 1911, xxix, p. 274.

³ HOSKINS: *this Journal*, 1912, xxix, p. 363.

on account of the ease with which blood pressure can be recorded, studies of the relation of the adrenals to the sympathetics can well be made on the vasomotor system.

Such investigations have been made by Strehl and Weiss,⁴ Young and Lehman,⁵ and later by Young⁶ alone. The method in each case was, in brief, after opening the abdominal cavity, to pass the ends of ligatures through the body wall on each side of the adrenal glands in such a way that by traction upon them the adrenal vessels should be occluded. Thus, with a minimal disturbance of the viscera, the discharge of the glands into the circulation could be stopped. Strehl and Weiss experimented upon rabbits, and Young and Lehman upon dogs. While blood pressure was being recorded, the ligatures were drawn tight; after ten to thirty minutes they were released. Strehl and Weiss reported that the ligation was succeeded by an immediate fall of pressure followed by a rise when the ligatures were removed. Young and Lehman, after occluding the vessels, observed but little fall, and that occurred very gradually. Upon releasing the ligatures, in three of their eight experiments, a decided rise of pressure occurred; in two, a slight rise; and in three no change. Young, in a repetition of the experiment, observed no significant fall of pressure for hours after tying off the glands.

Elliott⁷ and others have shown that circulating epinephrin is quickly destroyed. If therefore the adrenals by their secretion exert a constant tonic influence upon the sympathetics, ligating off the glands should result in an immediate fall of pressure. Whether or not a subsequent rise occurs after releasing the gland is of little significance. Checking the adrenal circulation might have resulted either in a long-continuing depression of the glands, such as results in the kidney when the renal vein is occluded, or in an accumulation of elaborated secretion. In either case removing the ligatures would not restore normal conditions.

In view of the conflicting results previously recorded, a repetition of the experiments seemed desirable, particularly since a number of

⁴ STREHL und WEISS: *Archiv für die gesammte Physiologie*, 1901, lxxxvi, p. 107.

⁵ YOUNG and LEHMAN: *Journal of physiology*, 1908, xxxvii, p. liv.

⁶ YOUNG, cited by VINCENT: *Ergebnisse der Physiologie*, 1910, ix, p. 579.

⁷ ELLIOTT: *Loc. cit.*, p. 444.

sources of experimental error are now recognized of which the earlier investigators were probably not cognizant. It is necessary in such experiments to start from, as nearly as possible, a normal epinephrin level. If under the influence of emotions, strong sensory stimulation, or asphyxia the adrenals were discharging an augmented secretion, any fall of pressure succeeding the ligation would be of doubtful significance. There would, in any case, be a fall of pressure at least *to normal*.

Our experiments were made upon eleven dogs. The effects of anæsthetics upon the adrenals being unknown, a variety of substances was used: urethane, urethane and ether, ether alone, and chloroform. The method of procedure in nine cases was essentially that of Strehl and Weiss. After inserting a cannula in the carotid artery the abdomen was opened by a median incision, and while the viscera were protected by warm towels, the ends of strong ligatures were inserted through the body wall in such a manner that by subsequent traction the circulation of each adrenal could be cut off. The abdomen was then closed. After waiting for some time for the immediate effects of the operation to pass off, the carotid cannula was connected with a recording manometer. By use of a slow extension kymograph a continuous graphic record of the whole experiment was secured. After blood pressure had been maintained for some minutes at a constant level, one ligature was drawn tight, then, after a three to five minute interval, the second also. For the reasons previously mentioned, a subsequent removal of the ligatures was regarded as useless. Post-mortem examinations showed that the ligatures had successfully occluded the adrenal vessels. In two instances the adrenals were dissected free and tied off by mass ligatures directly, the abdominal cavity remaining open.

In view of the evanescent nature of circulating epinephrin, the experiments were continued only from ten to thirty minutes. It was assumed that any direct results of adrenal deficiency would occur within that time. Indeed, in two instances, it was proven that under the experimental conditions a quantity of adrenalin injected in amount just sufficient to produce a demonstrable effect was quickly destroyed.

Our results agree consistently with those of Young and Lehman. In only one case after ligating off a gland was any fall of pressure observed. In this instance the ligature had inadvertently been placed diagonally so as probably to block enough splanchnic fibres to account

for the slight fall that occurred. Usually at the moment of tying the ligature there was a brief fluctuation of pressure, sometimes up and sometimes down, but the original level was quickly regained.

Since the ligation of both glands was without effect, any possible influence of accessory chromaffine tissue was ignored.

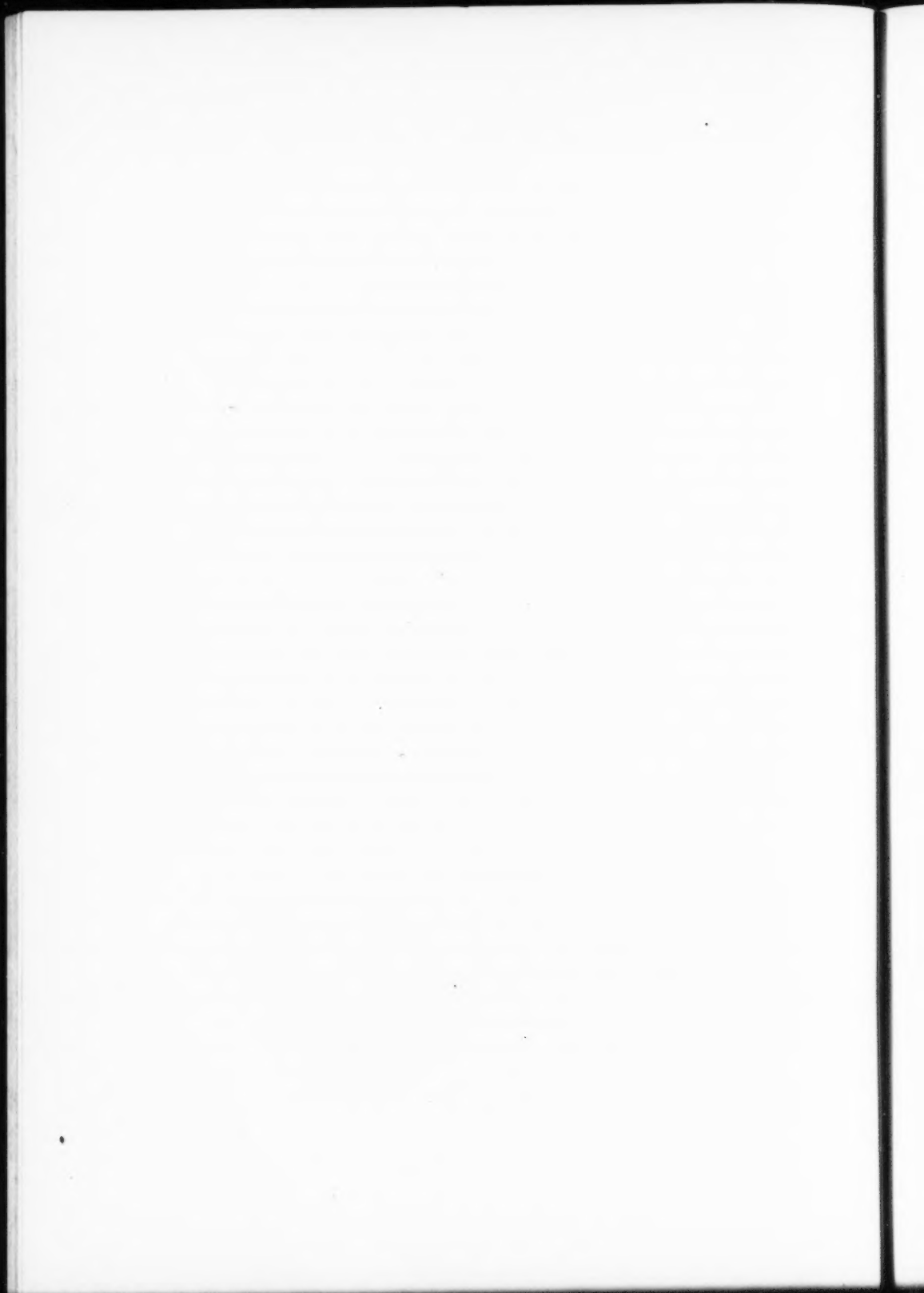
Our experiments, like those of previous investigators along this line, are not conclusive as regards normal blood pressure. It would be essential to know that the glands were secreting in a normal way at the time the ligatures were tied to permit absolute conclusions. If, owing either to anæsthesia or trauma, the glands were sufficiently depressed, the results would be without significance. It remains for subsequent research to exclude these possibilities.

Since, of the sympathetic fibres, the vasomotors are particularly sensitive to the effects of epinephrin, it seems justifiable to conclude that at least under the conditions of the experiments the assumed tonic influence of the adrenals upon the sympathetic system is non-existent.

Such an influence has been supposed to be shown by the fact that adrenal extirpation finally results in a circulatory failure. The facts, however, that circulating epinephrin is quickly destroyed, whereas the weakness following the removal of the glands develops hours or days afterwards, and that the asthenia is shared by skeletal muscle which has no sympathetic innervation, indicate that it is due rather to an interference with the nutritional processes in the tissue. In the present state of our knowledge speculation as to the exact nature of the interference is scarcely profitable.

SUMMARY.

In eleven dogs under anæsthesia ligating off the adrenal glands caused no immediate fall in blood pressure, although the animals were sensitive to minute doses of adrenalin and under the existing conditions injected epinephrin was quickly destroyed. The vasoconstrictor and supposedly other sympathetic fibres, therefore, had not been under a tonic influence exerted by the adrenal glands.



THE INFLUENCE UPON METABOLISM OF NON-OXIDIZABLE MATERIAL IN THE INTESTINAL TRACT.

BY FRANCIS G. BENEDICT AND LOUIS E. EMMES.

[Contribution from the Nutrition Laboratory of the Carnegie Institution of Washington.]

WHEN food is ingested, an increase in the oxygen consumption and carbon dioxide production is invariably noticed by all observers. At first it was believed that the presence of oxidizable material in the blood caused this increase in combustion, but later, since it was noted that frequently the increase occurred soon after taking meals, it was thought that the gain in metabolism might be due to the muscular work involved in the process of digestion. This latter idea was warmly advocated by Zuntz and v. Mering,¹ who found, by injecting directly into the blood stream nitrogenous as well as non-nitrogenous material, that there was essentially no increase in the oxygen consumption. They also found that when the same material was placed in the stomach there was an immediate gain in the oxygen consumption. This led to the belief that the increased metabolism noted after the ingestion of food was, in large part at least, due to the muscular activity involved in the processes of digestion.

That the muscular movements of the stomach and the intestinal tract play an important rôle in metabolism seemed to be substantiated by the experiments of Loewy,² who employed a dilute solution of sodium sulphate, provoking thereby a powerful peristaltic effect. In these experiments Loewy noted enormous increases in the oxygen consumption and carbon dioxide production. His results have been frequently cited, and undoubtedly have done much to establish the belief that the increased metabolism following the ingestion of food is in large part, if not wholly, due to the mechanical work of digestion. A critical

¹ ZUNTZ and VON MERING: *Pflüger's Archiv für die gesammte Physiologie*, 1877, xiv, p. 634; and 1883, xxxii, p. 173.

² LOEWY: *Pflüger's Archiv für die gesammte Physiologie*, 1888, xliii, p. 515.

examination of Loewy's protocols shows them decidedly deficient in information as to the muscular movements of the subjects during the tests.

In connection with a lengthy series of experiments in this laboratory on the influence of the ingestion of food upon metabolism, it seemed desirable to make some tests with sodium sulphate, determining the respiratory exchange by means of the respiration apparatus here employed. All of Loewy's experiments were carried out with the older form of Zuntz-Geppert respiration apparatus, and it was believed that, with a more recently developed apparatus and more modern views regarding the influence upon metabolism of minor muscular activity, the experiments should be repeated. Accordingly we have carried out a number of experiments with different individuals in which we have followed the details of experimentation outlined by Loewy, with the single exception that we have employed the respiration apparatus used in this laboratory instead of the Zuntz-Geppert apparatus. The very frequent reference to these experiments of Loewy as conclusive proof of the important part mechanical intestinal movement plays in the increased metabolism noted after the ingestion of food justifies the amplification of Loewy's earlier study.

While the introduction of a saline purgative would be provocative of intense peristaltic action, Professor W. B. Cannon of the Harvard Medical School thought that the use of some bulky material, which might perhaps involve the segmentation process to a larger extent than the use of sodium sulphate, would prove especially interesting. With such a material even greater muscular work would probably be produced than the simple peristalsis with sodium sulphate, which might result in a relatively few powerful peristaltic waves, while the process of segmentation would continue for a long period. Accordingly a series of experiments was included in the research in which agar-agar was ingested. This material furnishes bulk and has been shown to be almost undigested by the normal individual.

APPARATUS AND METHODS USED IN THE EXPERIMENTS.

The respiration apparatus used in these experiments was that regularly employed in this laboratory, which has been described in an earlier paper.³ The subjects were all normal individuals, some of

whom had previously been used for subjects of respiration experiments, while others were wholly unaccustomed to the apparatus. Each subject came to the laboratory in the morning without breakfast, having had no food for at least twelve hours. Previous to the experiment he lay upon a couch for a period varying from twenty to forty-five minutes in order to insure a condition of muscular rest and quietness, and the experiment was not begun until the pulse rate had reached a normal and constant level. The pulse rate was taken by means of a Bowles stethoscope placed over the apex beat of the heart, a long rubber transmission tube leading to the earpieces of an observer permitting records to be secured without the knowledge of the subject. In order to detect any extraneous movements or restlessness which might affect the metabolism, graphic records were made of the muscular quietness of the individual, these being obtained by means of tube pneumographs fastened about the chest and the thigh of the subject. With these pneumographs in place, it was absolutely impossible for the subject to move even his hand or his foot without a record being made on the smoked paper of the kymograph; it was thus practicable to determine whether or not the requisite degree of quietness had been obtained in order to secure a true measure of the possible changes in metabolism due to the sodium salt or the agar-agar.

In all, thirteen experiments were made, six with sodium sulphate and seven with agar-agar, eight different subjects being used. Each experiment consisted of a number of fifteen-minute periods with intervals of half to three quarters of an hour. Throughout the whole experiment the subject lay quietly upon a couch, except when the sodium sulphate or the agar-agar was administered. Before giving either material, two or three preliminary periods were obtained, these being continued until the results agreed satisfactorily. During the experimental periods the oxygen consumption and the carbon dioxide production were determined, and records made of the pulse and respiration rates and the muscular activity. In comparing the results of the experiments the pneumograph curves were first carefully examined, and any experiments showing extraneous muscular activity were rigidly excluded.

³ BENEDICT: this Journal, 1909, xxiv, p. 345. An elaborate description of the apparatus as now in use with several important modifications is soon to appear in the *Deutsches Archiv für klinische Medizin*.

Although these experiments were not planned primarily as a study of metabolism without food, the values for the carbon dioxide produced and the oxygen consumed represent probably the minimum metabolism for these men without food, lying awake and absolutely quiet; hence the statistics for body weight and height are worthy of record. These are given in Table I.

TABLE I.
STATISTICS OF AGE, HEIGHT, AND AVERAGE WEIGHT OF SUBJECTS.

Subject.	Age.	Height.	Weight without clothing.	Subject.	Age.	Height.	Weight without clothing.
	years	cm.	kilos.		years	cm.	kilos.
O. F. M.	24	171	86	F. P. R.	22	173	66
W. A. M.	23	183	78	J. J. C.	27	175	65
J. P. C.	23	169	74	H. H. A.	21	164	64
Dr. N. K. W.	35	166	68	Dr. P. R.	40	164	56

EXPERIMENTS WITH SODIUM SULPHATE.

In each experiment, after two or three experimental periods, the subject was given 15 gm. of crystallized sodium sulphate dissolved in 200 c.c. of water. The experiment continued with the ordinary intervals for three or four hours, a period somewhat longer than was usual in Loewy's experiments. The results of the six experiments are best presented in a series of tables which follow.

Experiment of February 13, 1911, with J. J. C. — This subject, although long accustomed to the use of the respiration apparatus, is not considered an ideal subject for respiration experiments owing to his inability to keep awake. Not only is the metabolism demonstrably lower under such conditions, but this particular subject is liable to suddenly move the head, open the mouth, or move an arm or leg while asleep, and thus vitiate the results. During this experiment it was necessary to speak to the subject continually to keep him awake, and frequently a loud-sounding bell was struck.

After taking the sodium sulphate, the subject was questioned as to any sensations in the bowels. No discomfort was noted in any experi-

mental period. At 4.30 P. M. there was a very copious loose movement of the bowels. The results of the experiment are given in Table II.

TABLE II.
EXPERIMENT WITH J. J. C. USING SODIUM SULPHATE.

Date and time.	Carbon dioxide elim'd per min.	Oxygen absorbed per min.	Respiratory quotient.	Increase in		Average pulse rate.	Average resp. rate.
				Carbon dioxide.	Oxygen.		
Feb. 13, 1911	c.c.	c.c.		per cent	per cent		
9.05 A. M.	179	226	0.79	62	19
9.30 A. M.	179	228	0.79	57	16
10.07 A. M.	181	222	0.82	59	17
Average	180	225
10.50 A. M. ¹	198	237	0.84	10.0	5.3	61	16
11.17 A. M.	195	217	0.90	8.3	-3.6	61	16
11.46 A. M.	193	224	0.86	7.2	-0.4	59	16
12.14 P. M.	199	219	0.91	10.6	-2.7	61	17
1.29 P. M.	188	237	0.79	4.4	5.3	61	17
2.35 P. M.	193	235	0.82	7.2	4.4	61	19
¹ Drank 15 gm. sodium sulphate in 200 c.c. of water at 10.42 A. M.							

Experiment of February 22, 1911, with W. A. M. — This subject, who was a medical student, co-operated in every way toward the success of the experiment, and hence no difficulty was experienced on account of muscular movements, going to sleep, etc. After preliminary tests to accustom the subject to the apparatus, two experimental periods showed constancy in results. The subject was then given the usual dose of sodium sulphate in 200 c.c. of water. During the period beginning at 12.25 P. M. he reported an almost uncontrollable desire to defecate. This became so strong that he defecated at 1.10 P. M. The results of the experiment are given in Table III.

Experiment of February 26, 1911, with O. F. M. — This subject, also a medical student, co-operated most intelligently in the experiment,

making an ideal subject. During the period beginning at 11.39 A. M. the desire to defecate became very strong, and he defecated at 11.56 A. M. The stool was very copious and watery. The results of the experiment are given in Table IV.

TABLE III.
EXPERIMENT WITH W. A. M. USING SODIUM SULPHATE.

Date and time.	Carbon dioxide elim'd per min.	Oxygen absorbed per min.	Respiratory quotient.	Increase in		Average pulse rate.	Average resp. rate.
				Carbon dioxide.	Oxygen.		
Feb. 22, 1911	c.c.	c.c.		per cent	per cent		
9.26 A. M.	209	262	0.80	65	7
9.53 A. M.	210	263	0.80	66	11
Average	210	263
10.41 A. M. ¹	214	259	0.82	1.9	-1.5	65	7
11.13 A. M.	217	275	0.79	3.3	4.6	66	9
11.47 A. M.	227	273	0.83	8.1	3.8	70	7
12.25 P. M.	223	272	0.82	6.2	3.4	68	9
12.52 P. M. ²	230	270	0.85	9.5	2.7	70	10
1.30 P. M.	228	272	0.84	8.6	3.4	67	6
¹ Took 15 gm. sodium sulphate in 200 c.c. water at 10.29 A. M. ² Defecated at 1.10 P. M.							

Experiment of March 20, 1911, with J. P. C. — As this subject was also studying medicine, he readily grasped the importance of hearty co-operation and proved his ability to aid successfully in the experiment by controlling the muscular movements.

In the period beginning 1.27 P. M. the desire to defecate was strong. This continued throughout the next period, and the subject finally defecated at 3.10 P. M. During these periods there was considerable griping pain. The subject reported 6 loose movements during the night following the experiment. The tabulated results are given in Table V.

Experiment of January 21, 1912, with H. H. A. — This subject had had a long experience with the respiration apparatus and consequently the experiment made with him was especially satisfactory as to mus-

cular relaxation. The experiment was uneventful, save that the subject noticed no intense desire to defecate, did not defecate while in the laboratory, and reported that the first defecation occurred at 5 P. M., this being followed by others at 9.00 and 9.30 P. M.; in no instance were the stools watery. The results are given in Table VI.

TABLE IV.
EXPERIMENT WITH O. F. M. USING SODIUM SULPHATE.

Date and time.	Carbon dioxide elim'd per min.	Oxygen absorbed per min.	Respiratory quotient.	Increase in		Average pulse rate.	Average resp. rate.
				Carbon dioxide.	Oxygen.		
Feb. 26, 1911	c.c.	c.c.		per cent	per cent		
8.47 A. M.	220	275	0.80	62	15
9.21 A. M.	210	275	0.76	60	16
Average	215	275
10.13 A. M. ¹	203	263	0.77	-5.6	-4.4	56	15
10.39 A. M.	200	251	0.79	-7.0	-8.7	56	16
11.12 A. M.	214	272	0.79	-.5	-1.1	59	15
11.39 A. M.	224	272	0.82	4.2	-1.1	61	15
12.17 P. M. ²	221	286	0.77	2.8	4.0	61	16
1.05 P. M.	205	275	0.75	-4.7	...	60	16
1.38 P. M.	225	268	0.84	4.7	-2.5	60	14
¹ Took 15 gm. sodium sulphate in 200 c.c. water at 9.55 A. M. ² Defecated at 11.56 A. M.							

Experiment of January 28, 1912, with F. P. R. — This subject had been used for previous respiration experiments, and his familiarity with the apparatus and the experimental routine made him particularly satisfactory as a subject. The experiment was eventful in no way, save that in the period beginning at 12.29 P. M. a great deal of rumbling was noticed in the bowels. Defecation occurred at 1.25 P. M. the stool being thin and watery. During the next period, *i. e.*, in that beginning at 1.56 P. M., there were further intestinal movements and

rumblings; these continued until the second defecation at 2.50 P. M. The results of the experiment are given in Table VII.

TABLE V.
EXPERIMENT WITH J. P. C. USING SODIUM SULPHATE.

Date and time.	Carbon dioxide elim'd per min.	Oxygen absorbed per min.	Respiratory quotient.	Increase in		Average pulse rate.	Average resp. rate.
				Carbon dioxide.	Oxygen.		
Mar. 20, 1911	c.c.	c.c.		per cent	per cent		
8.49 A. M.	186	56	19
10.16 A. M.	192	232	0.83	56	19
10.44 A. M.	181	226	0.80	55	19
11.17 A. M.	186	230	0.81	55	20
Average	186	229
12.07 P. M. ¹	188	227	0.83	1.1	0.9	54	19
12.32 P. M.	196	237	0.83	5.4	-3.5	54	17
1.27 P. M.	192	238	0.81	3.2	3.9	56	18
1.57 P. M.	197	247	0.80	5.9	7.9	55	..
2.41 P. M.	190	243	0.78	2.2	6.1	56	20
3.43 P. M. ²	189	... ³	...	1.6	...	54	18
4.11 P. M.	195	245	0.79	4.8	7.0	55	..

¹ Drank 15 gm. sodium sulphate in 200 c.c. water at 11.45 A. M.
² Defecated and urinated at 3.10 P. M.
³ "Oxygen lost. Subject took mouthpiece out before the valve was properly turned."

CONCLUSIONS REGARDING THE INFLUENCE OF SODIUM SULPHATE SOLUTIONS ON THE GASEOUS METABOLISM.

In all of the tables the increase (or decrease) in the percentage of oxygen and carbon dioxide is recorded for the periods following the administering of the sodium sulphate for comparison with the results of the periods prior to taking the purgative. In no case do we find an increase in either the carbon dioxide or the oxygen commensurate in

any way with the high values reported by Loewy, whose results are summed up in the following translation of a paragraph from his article:

"The size of the increase in the metabolism varies within relatively wide limits; the most marked results are found in experiment No. 2 with

TABLE VI.
EXPERIMENT WITH H. H. A. USING SODIUM SULPHATE.

Date and time.	Carbon dioxide elim'd per min.	Oxygen absorbed per min.	Respiratory quotient	Increase in		Average pulse rate.	Average resp. rate.
				Carbon dioxide.	Oxygen.		
Jan. 21, 1912	c.c.	c.c.		per cent	per cent		
9.07 A. M.	203	221	0.92	64	11
9.33 A. M.	202	228	0.89	67	12
10.07 A. M.	196	228	0.86	68	10
Average	200	226
11.01 A. M. ¹	185	223	0.83	- 7.5	- 1.3	67	13
11.41 A. M.	178	217	0.82	- 11.0	- 4.0	65	14
12.20 P. M.	188	225	0.84	- 6.0	- 0.4	76	11
1.05 P. M.	187	227	0.82	- 6.5	0.4	73	13
1.45 P. M.	184	240	0.77	- 8.0	6.2	72	14
2.30 P. M.	178	227	0.78	- 11.0	0.4	73	13

¹ Took 15 gm. sodium sulphate in 200 c.c. water at 10.42 A. M.

subject B and in experiment No. 9 with subject W, where, in one case after taking 15 grams and in another case 10 grams of sodium sulphate, the increase amounted both for carbon dioxide and oxygen to over 30 per cent. One dose of 15 grams produced in experiment No. 1 with Dr. C. an increase of about 15 per cent, and in experiment No. 10 with F, there was an increase in the carbon dioxide of about 8 per cent. The smallest effect was noticed in experiments Nos. 6 and 7 with student C; in the latter experiment there is an increase of 7 per cent and in the first, an increase of 7 per cent of carbon dioxide, while the oxygen values remained practically unaltered."

It can be seen from Tables II-VII that practically all of our experiments are in exactly the same category as were those made by Loewy with student C; the only conclusion that can be drawn from these experiments is, therefore, that when suitable precautions are taken for the control of the extraneous muscular activity the ingestion of

TABLE VII.
EXPERIMENT WITH F. P. R. USING SODIUM SULPHATE.

Date and time.	Carbon dioxide elim'd per min.	Oxygen absorbed per min.	Respiratory quotient.	Increase in		Average pulse rate.	Average resp. rate.
				Carbon dioxide.	Oxygen.		
Jan. 28, 1912.	c.c.	c.c.		per cent	per cent		
9.11 A. M.	191	210	0.91	50	12
10.16 A. M.	194	219	0.88	57	11
11.06 A. M.	184	215	0.86	54	11
11.41 A. M.	179	221	0.81	53	...
Average	187	216
12.29 P. M. ¹	189	218	0.87	1.1	0.9	52	11
1.08 P. M. ²	174	227	0.77	-7.0	5.1	51	...
1.56 P. M.	178	212	0.84	-4.8	-1.9	50	13
2.30 P. M.	186	222	0.84	-0.5	2.8	54	...
¹ Drank 15 gm. sodium sulphate in 200 c.c. water at 12.13 P. M. ² Defecated at 1.25 P. M., also at 2.50 P. M.							

15 gm. of sodium sulphate in 150 to 200 c.c. of water, notwithstanding the intense peristalsis frequently produced, does not materially increase the gaseous metabolism of the body as a whole; consequently, to cite the earlier experiments in which sodium sulphate was administered as an indication of the important effect of the intestinal movements upon the total metabolism, particularly with reference to the increased metabolism following the ingestion of food, is erroneous.

EXPERIMENTS WITH AGAR-AGAR.

Since apparently a large amount of muscular movement occurs in the intestines as the result of the presence of food materials of

considerable bulk, it was decided to make a series of experiments in which a relatively insoluble substance, capable of yielding stools of large bulk, could be introduced into the intestines. For this purpose use was made of the Japanese agar-agar, which was administered either dry or in the form of jellies of various consistencies containing different portions of the substance. It was found that these jellies did not rapidly give up their moisture, even when placed in water in an oven at body temperature.

The subjects found it very difficult to eat large amounts of agar-agar, either in the form of jelly or as dry material. When given dry, the agar-agar was in the form of a powder, or else chopped in small pieces or pulverized. In interpreting these experiments it is necessary, however, to consider that there is still some absorption of carbohydrate from this material, although Saiki⁴ has shown that the absorption is very imperfect; indeed, the property of these Japanese materials and especially of agar-agar to retain water and yield stools of large bulk has led to their being employed as therapeutic agents. In order to make the agar-agar jelly more palatable, 1 per cent by weight of Liebig's extract of beef was added.

It was necessary that the experiments with agar-agar should be of much longer duration than those with sodium sulphate, since unquestionably the progress of material out of the stomach would be much slower; experiments were therefore continued for six to eight hours. One great difficulty was experienced in conducting long experiments of this kind, particularly with subjects who were not used to the apparatus, in that they became very tired. Although the separate experimental periods are actually only fifteen minutes long, it is necessary for the subjects in both the periods and the intermissions to lie quietly upon the bed with the muscular activity reduced to a minimum; only in this way is it possible to avoid the incidental rise in metabolism due to any increase in muscular activity, such as raising the body, sitting up, talking, or any other excitement. Under these conditions of voluntary and forced muscular rest, the subjects frequently complained of being very tired,⁵ and undoubtedly this of itself may stimulate somewhat the metabolism.

⁴ SAIKI: *Journal of biological chemistry*, 1906, ii, p. 251.

⁵ JOHANSSON also finds it impossible to continue experiments with enforced muscular rest for more than one hour. *Skandinavisches Archiv für Physiologie*, 1908, xxi, p. 3.

Experiment of March 4, 1911, with J. J. C. — Although as a rule unsatisfactory as a subject, J. J. C. was the first on whom a test was made of the influence of agar-agar feeding on metabolism. All the

TABLE VIII.

EXPERIMENT WITH J. J. C. USING AGAR-AGAR.

Date and time.	Carbon dioxide eliminated.		Average pulse rate.	Average respiration rate.
	Per minute.	Increase.		
March 4, 1911	c.c.	per cent		
8.45 A. M.	198	..	71	17
9.17 A. M.	196	..	71	19
9.54 A. M.	195	..	64	17
10.22 A. M.	182	..	62	18
11.04 A. M.	206	..	63	19
11.37 A. M.	203	..	67	21
12.16 P. M.	197	..	64	19
1.06 P. M.	205	..	65	19
Average	198
2.11 P. M. ¹	208	5.1	62	20
2.45 P. M.	195	-1.5	58	19
3.21 P. M.	217	9.6	60	21
3.48 P. M.	202	2.0	59	20
4.25 P. M.	199	0.5	58	20
¹ Finished eating 219 gm. agar-agar and drinking 1 cup of black coffee at 2.05 P. M.				

oxygen values for this experiment were lost, owing to errors in technique; but the values for carbon dioxide are regarded as worthy of permanent record. The subject ate at 2.05 P. M. 219 gm. of a jelly made of 15 gm. of agar-agar, 750 c.c. boiling water, 7.5 gm. of Liebig's extract of beef, and about 8 gm. of table salt. The amount of dry agar-agar in the jelly eaten was computed to be 4.2 gm. Since he

found this a somewhat unpalatable jelly, he was also given a small cup of black coffee without sugar.

No peristaltic movements were felt by the subject. He defecated at 5 P. M. and again at 6 P. M.; while the movements were much softer than normal, they were in no sense watery. The results of the experiment are given in Table VIII.

TABLE IX.
EXPERIMENT WITH W. A. M. USING AGAR-AGAR.

Date and time.	Carbon dioxide elim'd per min.	Oxygen absorbed per min.	Respiratory quotient.	Increase in		Average pulse rate.	Average resp. rate.
				Carbon dioxide.	Oxygen.		
March 12, 1911	c.c.	c.c.		per cent	per cent		
8.25 A. M.	217	270	0.80	62	9
8.50 A. M.	218	254	0.86	64	9
9.27 A. M.	221	271	0.81	64	6
10.06 A. M.	202	264	0.76	63	12
Average	215	265
11.02 A. M. ¹	223	275	0.81	3.7	3.8	64	13
11.34 A. M.	219	265	0.83	1.9	0	64	10
12.01 P. M.	231	273	0.85	7.4	3.0	69	13
12.41 P. M.	231	268	0.86	7.4	1.1	70	11
1.56 P. M.	233	277	0.84	8.4	4.5	71	8

¹ Finished 223 gm. of jelly made from agar-agar at 10.52 A. M.

Experiment of March 12, 1911, with W. A. M. — This subject, who had previously been used in a sodium sulphate experiment, consented to serve also as subject in an agar-agar experiment. He succeeded in eating 223 gm. of jelly, containing 4.3 gm. of the dry agar-agar. In the last two periods the subject complained of a headache, so the experiment was discontinued. The results are given in Table IX.

Experiment of March 14, 1911, with J. J. C. — A change from the jelly to dry agar-agar was made in the second experiment with this

subject in the hope that larger amounts of the substance could be eaten. Of the dry pulverized material he ate 8.5 gm. with 300 c.c. of a 1 per cent solution of Liebig's extract of beef. The high values found in the first period after agar-agar are explainable only on the ground of stimulation caused by the muscular activity incidental to sitting

TABLE X.
EXPERIMENT WITH J. J. C. USING AGAR-AGAR.

Date and time.	Carbon dioxide elim'd per min.	Oxygen absorbed per min.	Respiratory quotient.	Increase in		Average pulse rate.	Average resp. rate.
				Carbon dioxide.	Oxygen.		
Mar. 14, 1911	c.c.	c.c.		per cent	per cent		
9.11 A. M.	198	227	0.87	66	14
9.48 A. M.	187	220	0.85	63	15
10.16 A. M.	196	225	0.87	63	14
Average	194	224
11.02 A. M. ¹	219	248	0.88	12.9	10.7	73	19
11.33 A. M.	202	225	0.90	4.1	0.4	64	17
12.07 P. M.	204	236	0.86	5.2	5.4	64	15
12.32 P. M.	202	231	0.88	4.1	3.1	60	15
1.11 P. M.	206	232	0.89	6.2	3.6	62	15
1.53 P. M.	210	247	0.85	8.2	10.3	62	15
3.04 P. M.	203	242	0.84	4.6	8.0	65	17
4.26 P. M.	206	260	0.79	6.2	16.1	64	17
¹ Took 8.5 gm. agar-agar at 10.45 A. M. with 300 c.c. of 1 per cent beef tea.							

up and drinking the hot beef tea. The pulse rate was abnormally high in this period, but in the next period had returned to its accustomed level. The last three periods were characterized by the great restlessness of the subject just before the periods began. The results of the experiment are given in Table X.

Experiment of March 19, 1911, with O. F. M. — The previous experience of this subject in a sodium sulphate experiment made him especially valuable for an agar-agar test. He was able to eat 15 gm.

of dry agar-agar with 300 c.c. of a 1 per cent solution of Liebig's extract of beef. During the last three periods the subject complained bitterly of backache, and was greatly relieved when the experiment was over. The results of the experiment are given in Table XI.

TABLE XI.
EXPERIMENT WITH O. F. M. USING AGAR-AGAR.

Date and time.	Carbon dioxide elim'd per min.	Oxygen absorbed per min.	Respiratory quotient.	Increase in		Average pulse rate.	Average resp. rate.
				Carbon dioxide.	Oxygen.		
March 19, 1911	c.c.	c.c.		per cent	per cent		
8.55 A. M.	207	261	0.79	55	15
9.33 A. M.	208	53	14
10.09 A. M.	184	246	0.75	51	14
10.50 A. M.	214	269	0.80	52	14
Average	203	259
11.53 A. M. ¹	207	263	0.79	2.0	1.5	54	15
12.28 P. M.	216	260	0.83	6.4	0.4	53	15
1.10 P. M.	224	10.3	...	53	14
1.42 P. M.	220	283	0.78	8.4	9.3	53	17
2.21 P. M.	230	283	0.81	13.3	9.3	55	18
2.54 P. M.	223	277	0.80	9.9	7.0	56	19
¹ Ate 15 gm. agar-agar with 300 c.c. of 1 per cent beef tea at 11.30 A. M.							

Experiment of April 17, 1911, with J. P. C — This subject had also been used in a sodium sulphate experiment. Unfortunately he was able to eat only 5.8 gm. of agar-agar, with 200 c.c. of 1 per cent Liebig's extract solution. He defecated at 2.30 P. M.; in the last period he was very restless and at 3.37 P. M. defecated a second time. The results of the experiment are given in Table XII.

Experiment of May 8, 1911, with Dr. N. K. W. — The most hearty co-operation was accorded us by Dr. W., who took a great interest in the experiment. Although wholly unused to the apparatus, he

immediately adjusted himself to the experimental routine in a most successful manner. Of special note is the fact that he was able to eat 23.28 gm. of dry agar-agar, using water instead of beef tea to assist in swallowing it. The intelligent comments of the subject aided materially in the interpretation of the results of the experiment.

TABLE XII.

EXPERIMENT WITH J. P. C. USING AGAR-AGAR.

Date and time.	Carbon dioxide elim'd per min.	Oxygen absorbed per min.	Respiratory quotient.	Increase in		Average pulse rate.	Average resp. rate.
				Carbon dioxide.	Oxygen.		
April 17, 1913	c.c.	c.c.		per cent	per cent		
9.09 A. M.	192	215	0.90	55	18
9.40 A. M.	178	202	0.88	50	19
10.11 A. M.	185	214	0.87	51	19
Average	185	210
11.25 A. M. ¹	185	222	0.83	0	5.7	54	19
11.59 A. M.	193	221	0.87	4.3	5.2	55	20
12.30 P. M.	187	226	0.83	1.1	7.6	52	19
1.01 P. M.	195	223	0.87	5.4	6.2	54	19
1.30 P. M.	185	225	0.82	0	7.1	52	19
2.05 P. M. ²	185	0	...	55	19
2.55 P. M.	189	227	0.83	2.2	8.1	51	21

¹ Took 5.8 gm. agar-agar with 200 c.c. of 1 per cent beef tea (Liebig's) at 10.50 A. M.
² Defecated at 2.30 P. M.

An increased peristalsis was noted in the periods beginning at 11.11 A. M., 12.19 P. M., and 12.58 P. M. During the period beginning at 1.33 P. M. he felt faint and the abdomen was sore. During the period beginning at 2.46 P. M. he became nervous, so sat up for a time after the period, urinating at 3.20 P. M. During the later periods he said that he felt tired. After leaving the laboratory his report was that he "drank six large glasses of water, as my mouth was very dry." This suggests that the water-retaining property of this large amount of

agar-agar may have been accountable for the dryness. The results of the experiment are given in Table XIII.

Experiment of October 19, 1911, with Dr. P. R. — Having had an extensive experience not only in metabolism experiments, but particu-

TABLE XIII.

EXPERIMENT WITH DR. N. K. W. USING AGAR-AGAR.

Date and time.	Carbon dioxide elim'd per min.	Oxygen absorbed per min.	Respiratory quotient.	Increase in		Average pulse rate.	Average resp. rate.
				Carbon dioxide.	Oxygen.		
May 8, 1911	c.c.	c.c.		per cent	per cent		
9.07 A. M.	203	228	0.89	50	13
9.32 A. M.	191	215	0.89	49	16
10.10 A. M.	194	221	0.88	50	16
Average	196	221
11.11 A. M. ¹	201	233	0.86	2.6	5.4	51	16
11.46 A. M.	201	221	0.91	2.6	0	52	19
12.19 P. M.	211	234	0.90	7.7	5.9	53	18
12.58 P. M.	205	234	0.88	4.6	5.9	55	17
1.33 P. M.	189	242	0.78	-3.6	9.5	52	17
2.08 P. M.	195	246	0.79	-0.5	11.3	54	17
2.47 P. M.	220	256	0.86	12.2	15.8	57	18
3.59 P. M.	210	249	0.84	7.1	12.7	55	17
4.27 P. M.	193	263	0.74	-1.5	19.0	55	18
¹ Ate 23.28 gm. agar-agar and 400 c.c. cold water 10.37-10.50 A. M.							

larly with the respiration apparatus, Dr. R. kindly consented to be a subject in this series of experiments. A commercial preparation of agar-agar was used, which, however, was essentially the common laboratory grade material. Dr. R. made an ideal subject, being of a remarkable temperament, in that his muscular control was complete and he was without nervousness. He also had a thorough understand-

ing of the importance of the experiment, and particularly of the importance of muscular relaxation. This subject took 17.71 gm. of dry agar-agar, with one glass (200 c.c.) of water.

TABLE XIV.
EXPERIMENT WITH DR. P. R. USING AGAR-AGAR.

Date and time.	Carbon dioxide elim'd per min.	Oxygen absorbed per min.	Respiratory quotient.	Increase in		Average pulse rate.	Average resp. rate.
				Carbon dioxide.	Oxygen.		
Oct. 9, 1911	c.c.	c.c.		per cent	per cent		
9.45 A. M.	163	199	0.82	63	14
10.11 A. M.	151	193	0.78	60	14
10.36 A. M.	160	189	0.85	60	14
11.04 A. M.	153	193	0.79	59	15
Average	157	194
12.07 P. M. ¹	160	196	0.82	1.9	1.0	58	14
12.44 P. M.	151	207	0.73	-3.8	6.7	59	14
1.40 P. M.	153	195	0.78	-2.6	0.5	59	15
2.15 P. M.	155	189	0.82	-1.3	-2.6	61	15
3.13 P. M.	152	195	0.78	-3.2	0.5	57	16
3.49 P. M.	158	204	0.78	0.6	5.2	60	15
4.20 P. M.	158	204	0.78	0.6	5.2	61	16
¹ After 17.71 gm. agar-agar with 200 c.c. water at 11.29-11.40 A. M.							

The whole experiment was ideal, as there was an absolute absence of headache, backache, and restlessness. Such an experiment, in which the subject lies absolutely quiet from 9.00 A. M. to 4.35 P. M. aside from the ten minutes occupied in eating the agar-agar, is rather remarkable. Without this complete co-operation on the part of the subject, such a long experiment would be absolutely impossible. The results are given in Table XIV.

DISCUSSION OF THE RESULTS WITH THE INGESTION OF
AGAR-AGAR.

In considering the results of the experiments with agar-agar, it will be necessary to bear in mind the fact that the long-continued experiments made necessary by the relatively slow action of the agar-agar in the intestinal tract are especially fatiguing to the subject. It will be noticed that whenever the subjects complained of headache, backache, or weariness, the metabolism is increased; and it is reasonable to attribute such an increase directly to these extraneous factors. The greatest increase in metabolism noted was that in the last period of the experiment with Dr. N. K. W., in which there was a gain of 19 per cent in the oxygen consumption. Inasmuch as this was not accompanied by an increase in the production of carbon dioxide, and we have no reason to assume that there was a marked change in the character of the metabolism, it is highly probable that this value was due to an error in technique caused by the restlessness of the subject; although the pneumograph curves did not show any great muscular movements, nevertheless, movements of the head may have caused a leak around the nosepieces which caused the increase. It is certainly true that the results of the last three periods with Dr. N. K. W. are open to the objection that the conditions were distinctly abnormal. Similarly, in the experiment with O. F. M., the subject complained steadily of backache during the last three periods, while in the experiment of March 14, J. J. C. was very restless. Making allowance for these extraneous factors, it is seen that with the majority of subjects there is no measurable increase in metabolism that can be rightly attributed to increased segmentation on account of the ingestion of agar-agar. Fortunately the particularly valuable and satisfactory experiment with Dr. P. R. of October 19, 1911, was carried to a conclusion without such factors as headache, backache, restlessness, or extraneous muscular activity of any kind. Under these ideal conditions the maximum oxygen increase amounted to but 6.7 per cent.

It would appear, therefore, from the results obtained in this series of experiments, that the movement along the intestinal tract of a large bulk of material, such as agar-agar taken either in jelly form or with water, is accomplished without the expenditure of any consider-

able or measurable amount of energy; it would therefore appear that the "work of digestion," in so far as either peristalsis or possibly the segmentation process is concerned, cannot be of sufficient moment to play an important rôle or to explain in any degree the marked rise in metabolism so frequently noted after the ingestion of various food materials.

THE INFLUENCE ON THE RESPIRATORY EXCHANGE OF VARYING AMOUNTS OF CARBOHYDRATE IN THE DIET.

BY FRANCIS G. BENEDICT AND HAROLD L. HIGGINS.

[*Contribution from the Nutrition Laboratory of the Carnegie Institution of Washington, Boston, Massachusetts.*]

THE significance of the respiratory quotient in indicating the character of the katabolism has been pointed out by Zuntz and his associates. Simultaneously with the improvement of technique and methods for the determination of oxygen absorption by the body, and with the more thorough understanding of factors which are likely to disturb a properly conducted respiration experiment, the respiratory quotient has in recent years come to be of increased practical importance and thus to receive considerable attention.

It is commonly known that soon after the ingestion of carbohydrates, especially the soluble carbohydrates, there is a marked rise in the respiratory quotient indicating a katabolism of sugar and possibly the conversion of sugar to fat. The prevailing view has been, however, that after active digestion has ceased — probably in six hours and at least in twelve hours — the storage of body carbohydrate has essentially reached constancy, to be then gradually depleted as inanition proceeds. This view has been strongly supported by the statement of Magnus-Levy¹ to the effect that he was unable to increase the respiratory quotient by feeding a man enormous quantities of carbohydrates for at least two days. On the other hand, Benedict, Emmes, and Riche² in this laboratory, using a small respiration apparatus,³ found that when the evening meal contained a large amount of carbohydrate the respiratory quotient on the following morning,

¹ MAGNUS-LEVY: *Pflüger's Archiv für die gesammte Physiologie*, 1894, lv, p. 25.

² BENEDICT, EMMES, and RICHE: *this Journal*, 1911, xxvii, p. 383.

³ BENEDICT: *this Journal*, 1909, xxiv, p. 345.

at least twelve hours after the last meal, was higher than when the preceding meal was poor in carbohydrates. The larger combustion of body carbohydrates being interpreted as indicating a larger supply, it seemed proved that diet must play an important rôle in glycogen supply. The importance of body glycogen in connection with inanition, muscular work, and probably athletic training, as well as the great physiological interest in the amount and variation of body glycogen (undoubtedly much greater in percentage than the variation of body fat or protein), has led us to elaborate and continue the previous research.

Instead of restricting our study to the evening or last preceding meal, we have followed the diet of the whole day previous. Furthermore, instead of limiting the scope to merely the question of "large" or "small" amounts of carbohydrate, we have quantitatively fixed the amount of carbohydrate, and also the protein and fat contents and the fuel value, in each of several diets; thus we were able to determine the relation between the carbohydrate content of the diet and the respiratory quotient the next morning without food. As the condition of the supply of body carbohydrate previous to beginning the day's diet would probably affect the results, we have given the same diet several days — generally four — in succession, observing the respiratory exchange before beginning the diet and after each successive day of the study. In this manner we not only obtained what may be called the true respiratory exchange resulting from the diet, but we also obtained an index of the changes in carbohydrate content of the body leading up to this condition. In order not to place too much weight upon results which might be due to the idiosyncrasies of a single individual, and also to find if any such idiosyncrasies existed, a number of subjects were included in the study, — seven in all.

Six different diets were experimented with; the daily composition of each diet is given in Table I. In the experiments with any one diet the composition for each day varied not more than 5 gm. for the protein, fat, or carbohydrate, and not more than 30 calories in total energy. Except in a few instances, the same foods were taken at the same time, the menu for each diet being fixed. The foods chosen were easily obtained and prepared and were of approximately constant composition. As a rule, the composition and caloric value of the materials

were taken from Atwater and Bryant's tables,⁴ but in a few cases analyses were made of the individual foods.

In making up the diet care was taken to have it as palatable as possible on the principle that food not relished might lead to unsatisfactory results. With this in view, also, several minor changes were occasionally made to suit the tastes of the different men, as, for

TABLE I.
COMPOSITION OF THE DIETS.

Diet No.	Protein.	Fat.	Carbohydrates (amount as starch in parentheses).	Calories.
1	gm. Varying	gm. Varying	gm. 0	About 2400
2	105	230	100 (48)	3000
3	105	235	125 (60)	3100
4	105	190	200 (120)	3000
5	105	100	400 (235)	3000
6	100	24	600 (200)	3050

instance, in the experiments with one subject lemon juice and sugar were substituted for oranges. There was no consensus of opinion in favor of any one of the various diets, some subjects preferring the high and some the low carbohydrate diet. The carbohydrate-free diet, however, was found rather trying by both the subjects who used it, but in no other case was the diet reported as objectionable, even after four days.

Usually the food was given in three meals, at 8.45 A. M., 1 P. M., and 5 P. M., respectively. These meals were approximately equal in amount, although probably the evening meal was in each case somewhat the largest. In the experiments with diet No. 3, two meals were given at 10.30 A. M. and 6 P. M., respectively, with a light lunch at 5 P. M.

It will be observed that diets Nos. 2, 3, 4, 5, and 6 were chosen to

⁴ ATWATER and BRYANT: United States Department of Agriculture, Office of Experiment Stations, Bulletin 28, 1906.

give constant caloric value and protein content, with varying relative amounts of carbohydrates and fats. Thus, by comparing the results with those obtained with diet No. 1, we have the opportunity of observing the effects of the varying amounts of carbohydrate.

A fundamentally important factor in experiments of this kind is the hearty and intelligent co-operation of the subject. We were very

TABLE II.
STATISTICS OF SUBJECTS.

Subject.	Diets.	Height.	Weight.	Subject.	Diets.	Height.	Weight.
		cm.	kilos.			cm.	kilos.
H. L. H.	1, 3, 6	172	61.5	W. G. J.	4, 5	175	60.0
H. H. A.	1, 5, 6, 6	166	61.0	D. M.	2	171	64.0
F. P. R.	2, 5, 6	173	64.5	E. T. W.	4	169	58.0
C. B. S.	2, 5, 6	182	70.0

fortunate in securing as subjects, in addition to one of us, a number of students from the Harvard Medical and Harvard Dental Schools. These men all took a keen interest in the experimentation and co-operated in every way to make the study a success, especially by insuring strict dietetic control in that no food was taken other than that actually recorded in the diet. The subjects were all young men, twenty-one to thirty years of age, and in good health. Data regarding their height without shoes, and naked body weight, also the diets given each subject, may be found in Table II.

Respiration experiments were made on the morning on which the subject began his diet and on the morning following each diet day, the apparatus used being essentially the same as that employed in the previous study. All of the experiments were made "nüchtern," *i. e.*, twelve hours after the preceding meal, with the conditions so fixed that the results would be comparable with one another and with the numerous other experiments which have been conducted in this laboratory for the past few years. The subject reached the laboratory about 7 A. M., and lay upon a couch quietly for approximately thirty minutes before the beginning of the experiments, so that his metabolism might become adjusted to the new position; this adjustment

was generally taken as complete when the pulse had fallen to a constant level. Three fifteen-minute respiration experiments were, as a rule, made on each day. Nosepieces⁵ were used in all cases, the lips being closed with surgeon's plaster to prevent the subject from inadvertently breathing through his mouth and thus introducing an error. To compare satisfactorily the respiratory exchange, and especially the index of total metabolism commonly accepted as the most reliable, *i. e.*, the oxygen consumption, the subjects kept perfectly still during the experiments, making practically no voluntary muscular movements. After observing the disturbing effect of fever in one series of experiments, the sublingual body temperature was taken at least once during each morning's experimenting. Observations of the pulse rate were also obtained in each experiment without the knowledge of the subject by means of a Bowles stethoscope with especially long leads. These pulse records are of unusual interest in view of the fact that the total metabolism seems to have an almost direct relationship to the pulse rate.

Each subject adhered essentially to his daily routine of life so as to secure a fair constancy of muscular work throughout each day, since unusual muscular work would affect the quantity of body carbohydrate consumed. To obtain a rough measure of the activity, the subjects as a rule carried a pedometer, which served to record all up and down movements of the body, the pedometer readings giving an approximate comparison of the activity of the subject on different days.

The respiration experiments of a morning agreed, as a rule, very closely. We have therefore felt justified in using in the following tables the averages for each day in view of the fact that over 85 per cent of the individual experiments differed from the daily mean by only 4 per cent or less, and over 50 per cent of the experiments by 2 per cent or less.

Diet No. 1, carbohydrate-free. — The experiments with diet No. 1 were made in connection with another research, but the results are given here, in Table III, to show the general trend of the respiratory quotient with a diet which was carbohydrate-free. The respiratory quotient fell very rapidly, reaching a minimum on the second day

⁵ BENEDICT: *Loc. cit.*, p. 364.

and remaining at a low value on the subsequent days, thus indicating sharply the almost complete absence of carbohydrate metabolism. In these experiments undoubtedly the acidosis affected the quotient. The diet varied greatly and was not accurately measured, but great care was taken that no carbohydrate was eaten.

TABLE III.
DIET NO. 1, CARBOHYDRATE-FREE.

Subject.	Date.	Day of diet.	Carbon dioxide elim'ted per min.	Oxygen absorbed per min.	Respiratory quotient.	Average pulse rate.	Body temperature.
H. L. H.	1911	Preliminary	c.c. 198	c.c. 237	0.84	..	F°
	Sept. 7	2d	200	299	.67	84
	Sept. 8	3d	197	280	.71	75
	Sept. 9	4th	195	272	.72	72
	Dec. 27	Preliminary	201	224	.90	78	98.2
H. H. A.	Dec. 28	1st	190	258	.74	83	98.7
	Dec. 29	2d	179	258	.70	74	97.9
	Dec. 30	3d	174	236	.74	71
	Dec. 31	4th	173	242	.71	79	97.7
	1912 Jan. 1	5th	164	239	.69	79	97.6

Diet No. 2, 100 gm. carbohydrates (3000 calories).—

8.45 A. M. 300 c.c. milk and cream, 2 boiled eggs, 10 gm. butter.

1.00 P. M. 300 c.c. milk and cream, scrambled eggs (2 eggs, 25 gm. butter, 75 c.c. milk and cream), 80 gm. peanuts (edible portion).

6.00 P. M. 280 c.c. milk and cream, 110 gm. canned salmon steak, 72 gm. lettuce, 10 gm. olive oil, 60 gm. bread, 21 gm. butter.

The "milk and cream" consisted of a mixture of 455 c.c. milk and 500 c.c. standard cream (18.5 per cent fat). The carbohydrate of the diet was divided among the meals into 15 gm. in the morning, 37 gm. at noon, and 48 gm. in the evening. The results of the experiments are given in Table IV. The respiratory quotient fell rapidly from a normal value of 0.81 or 0.82 to approximately 0.75, which is essentially a fasting quotient.

TABLE IV.

DIET No. 2, 100 GM. CARBOHYDRATES (3000 CALORIES).

Subject.	Date.	Day of Diet.	Carbon dioxide elim'ted per min.	Oxygen absorbed per min.	Respiratory quotient.	Average pulse rate.	Pedometer records.
	1911		c.c.	c.c.			miles
D. M.	Oct. 24	Preliminary	188	231	0.81	55	..
	Oct. 25	1st	203	263	.77	63	7½
	Oct. 26	2d	195	245	.79	69	4½
	Oct. 27	3d	186	236	.79	63	4½
	Oct. 28	4th	181	243	.74	60	4½
C. B. S.	Oct. 31	Preliminary	202	247	.82	64	..
	Nov. 1	1st	193	257	.75	65	5½
	Nov. 2	2d	193	265	.73	71	6½
	Nov. 3	3d	197	258	.76	66	6½
	Nov. 4	4th	193	261	.74	65	4½
F. P. R.	Nov. 1	Preliminary	179	217	.82	64	..
	Nov. 2	1st	173	236	.73	55	..
	Nov. 3	2d	175	234	.75	57	..
	Nov. 4	3d	173	239	.72	57	5
	Nov. 5	4th	174	240	.73	58	7½

Diet No. 3, 125 gm. carbohydrate (3100 calories). —

10.30 A. M. 335 c.c. milk, 2 boiled eggs, 25 gm. butter, 88 gm. peanuts (edible portion).

5.00 P. M. 250 c.c. milk.

6.00 P. M. 360 c.c. milk, 210 c.c. cream, 110 gm. canned salmon steak, 72 gm. lettuce, 56 gm. olive oil, 75 gm. bread, 31 gm. butter.

The cream contained 18.5 per cent fat. Approximately 38 gm. of carbohydrate were taken at 10.30 A. M., 12 gm. at 5 P. M., and 75 gm. at 6 P. M. In this series of experiments, which was the first carried out, a slightly different method of experimenting was used in that the respiration experiments were not made until 8 A. M. and the food was divided

into two meals instead of three. The results are given in Table V. Only one subject was tested on this diet, but here again the results indicate a fall in the quotient to about 0.75. The preliminary higher quotient of 0.81 indicates a previous diet containing more than 125 gm. of carbohydrate per day.

TABLE V.
DIET NO. 3, 125 GM. CARBOHYDRATES (3100 CALORIES).

Subject.	Date.	Day of diet.	Carbon dioxide elim'ted per min.	Oxygen absorbed per min.	Respiratory quotient.	Average pulse rate.	Pedometer record.
H. L. H.	1911 Sept. 20	Preliminary	c.c. 214	c.c. 265	0.81	78	Miles ..
	Sept. 21	1st	208	280	.74	78	9 $\frac{3}{4}$
	Sept. 22	2d	199	263	.76	72	9 $\frac{3}{4}$
	Sept. 23	3d	195	268	.73	74	9 $\frac{3}{4}$
	Sept. 24	4th	209	275	.76	81	9 $\frac{3}{4}$

Diet No. 4, 200 gm. carbohydrates (3000 calories). —

8.45 A. M. 300 c.c. modified milk, 2 boiled eggs, 75 gm. bread, 18 gm. butter.

1.00 P. M. 300 c.c. modified milk, 5 Graham crackers, 71 gm. peanuts (edible portion), 1 orange (*ca.* 130 gm.).

6.00 P. M. 300 c.c. modified milk, 110 gm. canned salmon steak, 72 gm. lettuce, 34 gm. olive oil, 75 gm. bread, 19 gm. butter, 8 gm. sugar, 1 orange (*ca.* 130 gm.).

The modified milk used was made up of 700 c.c. milk and 200 c.c. cream (18.5 per cent fat). The approximate division of carbohydrates was 54 gm. at 8.45 A. M., 72 gm. at 1 P. M., and 74 gm. at 6 P. M. The results of the experiments are given in Table VI. There is no obvious explanation of the marked fall in the respiratory quotient on the last day of the diet of each of the subjects, but it is to be presumed that the 200 gm. of carbohydrates were insufficient to maintain so high a respiratory quotient as 0.83 or 0.81, although it may be seen that the drain upon the previous supply was not so great as with 100 gm. of carbohydrate.

TABLE VI.
DIET NO. 4, 200 GM. CARBOHYDRATES (3000 CALORIES).

Subject.	Date.	Day of diet.	Carbon dioxide elim'ted per. min.	Oxygen absorbed per min.	Respiratory quotient.	Average pulse rate.	Pedometer record.
W. G. J.	1911 Oct. 17	Preliminary	c.c. 213	c.c. 255	0.84	70	Miles ...
	Oct. 18	1st	214	258	.83	73	7 $\frac{1}{4}$
	Oct. 19	2d	212	256	.83	69	7 $\frac{1}{4}$
	Oct. 20	3d	205	245	.84	69	7 $\frac{1}{2}$
	Oct. 21	4th	198	253	.78	66	8 $\frac{1}{2}$
E. T. W.	Oct. 19	Preliminary	178	218	.82	72	...
	Oct. 20	1st	170	213	.80	65	10
	Oct. 21	2d	174	216	.81	69	10
	Oct. 22	3d	161	211	.76	65	11 $\frac{1}{2}$

Diet No. 5, 400 gm. carbohydrates (3000 calories). —

8.45 A. M. 1 boiled egg, 150 gm. bread, 15 gm. butter, 50 gm. honey, 220 c.c. milk.

1.00 P. M. 71 gm. peanuts (edible portion), 100 gm. bread, 15 gm. butter, 25 gm. honey, 235 c.c. milk, 1 orange (*ca.* 150 gm.).

6.00 P. M. 110 gm. canned salmon steak, 100 gm. bread, 10 gm. butter, 72 gm. lettuce, 1 package candied popcorn (*ca.* 100 gm.), 20 gm. sugar as lemonade (5 gm. lemon juice), 1 orange (*ca.* 150 gm.).

The milk was not modified. Approximately 125 gm. of carbohydrate were taken at 8.45 A. M., 110 gm. at 1 P. M., and 165 gm. at 6 P. M. The results of the experiments are given in Table VII. On the second, third, and fourth days of the diet the subject H. H. A. had a cold and developed a light fever, his sublingual temperature averaging about 100° F. The results for these days are therefore abnormal, and do not represent the effects of the diet. They are included, however, to indicate the increased metabolism with fever as shown by the oxygen consumption.⁶ On the third day of the diet the subject W. G. J.

⁶ Compare CARPENTER and BENEDICT: this Journal, 1909, xxiv, p. 227.

developed a septic hand. The results of this day were also undoubtedly affected by the fact that the subject ran in coming to the laboratory in order to arrive in time for the experiment. These two causes doubtless raised the body temperature and pulse rate and possibly affected the respiratory exchange.

DIET NO. 5, 400 GM. CARBOHYDRATES (3000 CALORIES).

Subject.	Date.	Day of diet.	Carbon dioxide elim'ted per min.	Oxygen absorbed per min.	Respiratory quotient.	Average pulse rate.	Pedometer record.	Body temperature.
	Nov. 1911		c.c.	c.c.			Miles.	F. ^o
H. H. A.	7	Preliminary	173	194	.89	74
	8	1st	163	208	.78	72	7 $\frac{3}{4}$...
	9	2d	192	239	.80	86	6 $\frac{1}{4}$	100.6 ¹
	10	3d	184	239	.77	89	5 $\frac{3}{8}$	99.5 ¹
	11	4th	188	238	.79	94	7 $\frac{5}{8}$	99.6 ¹
F. P. R.	7	Preliminary	170	218	.78	57
	8	1st	172	215	.80	55	7	...
	9	2d	179	212	.84	54	5 $\frac{3}{8}$...
	10	3d	180	212	.85	54	4 $\frac{7}{8}$	97.6
	11	4th	186	213	.87	56	6	97.4
W. G. J.	14	Preliminary	207	252	.82	70	..	98.0
	15	1st	215	258	.83	76	6 $\frac{5}{8}$	98.6
	16	2d	216	257	.84	71	7 $\frac{5}{8}$	98.0
	17	3d	225	268	.84	90	12 $\frac{5}{8}$	99.0 ²
C. B. S.	14	Preliminary	191	239	.80	65	..	97.2
	15	1st	192	245	.78	64	6 $\frac{1}{4}$	97.8
	16	2d	188	239	.79	66	7 $\frac{1}{4}$	97.6
	17	3d	188	247	.76	63	6	97.5
	18	4th	196	243	.81	68	6 $\frac{3}{4}$	97.6

¹ Fever. ² Septic hand.

Unfortunately the results of these three series of experiments were vitiated by the febrile condition of H. H. A. and the septic hand of W. G. J.; furthermore those with the other two subjects are not coincident in that with F. P. R. the respiratory quotient steadily rose as the experiment progressed, while with subject C. B. S. the quotient was but little altered by the diet. The fact that the results with W. G. J. approach nearer to those of F. P. R. seems to indicate that about 0.83 or 0.84 is an approximately normal respiratory quotient for this diet.

Diet No. 6, 600 gm. carbohydrates (3050 calories).—More changes were made in the composition of this diet than of any other, but the total amounts of the fundamental constituents were unchanged. On December 5, with C. B. S. and H. H. A. (I), a large amount of the sugar was given in cranberry sauce; but as the 677 gm. of cranberry sauce eaten proved to be too large an amount for one day, it was omitted thereafter; so much cranberry sauce seemed also to produce a marked diuresis. In the two diets beginning December 19 some of the sugar at the noon meal was replaced by about 185 gm. of cranberry sauce. The general composition of the diet was as follows:

8.45 A. M. 150 gm. bread, 50 gm. honey, 500 c.c. fat-free modified milk, 30 gm. sugar.

1.00 P. M. 150 gm. bread, 50 gm. honey, 1 egg, 2 oranges (*ca.* 175–200 gm. each), 57 gm. sugar.

6.00 P. M. 50 gm. bread, 88 gm. canned salmon steak, 72 gm. lettuce, 400 c.c. fat-free modified milk, 1 package popcorn (*ca.* 100 gm.), 1 orange (*ca.* 175–200 gm.), 47 gm. sugar.

The milk was skimmed milk, 900 gm. of which were modified with 30 gm. of milk sugar. Especial care was taken in apportioning the diet so that in each of the meals there were 200 gm. of carbohydrate.

The results of the experiments are given in Table VIII. With this diet there was clearly a higher respiratory quotient than with the preceding diets. The general average of the respiratory quotients was about 0.87, with on the whole but little deviation from this figure. With two subjects, F. P. R. and H. H. A. (II), it required two days for the respiratory quotient to reach this level. The drop in the respira-

TABLE VIII.

DIET No. 6, 600 GM. CARBOHYDRATES (3050 CALORIES).

Subject.	Date.	Day of diet.	Carbon dioxide elim'ted per min.	Oxygen absorbed per min.	Respiratory quotient.	Average pulse rate.	Pedometer record.	Body temperature.
	Dec 1911		c.c.	c.c.			Miles	Fo.
C. B. S.	5	Preliminary	210	250	.84	63	..	97.5
	6	1st	207	238	.87	62	...	97.9
	7	2d	216	245	.88	63	..	97.9
	8	3d	213	244	.87	66	..	98.1
	9	4th	210	235	.89	60	..	97.4
H. H. A. (I)	5	Preliminary	189	211	.90	64	..	96.9
	6	1st	177	205	.86	62	5 $\frac{7}{8}$	96.8
	7	2d	185	206	.90	68	5 $\frac{5}{8}$	97.0
	8	3d	181	208	.87	67	6	97.4
	9	4th	176	210	.84	68	7	97.0
H. H. A. (II)	19	Preliminary	181	214	.85	64	..	97.0
	20	1st	177	225	.79	60	6 $\frac{1}{8}$	96.7
	21	2d	181	220	.82	70	6 $\frac{1}{4}$	96.8
	22	3d	179	208	.86	62	5 $\frac{5}{8}$	96.7
	23	4th	189	217	.87	70	7 $\frac{3}{8}$	97.0
F. P. R.	19	Preliminary	174	227	.77	59	..	97.4
	20	1st	173	213	.81	57	8 $\frac{3}{8}$	97.1
	21	2d	178	208	.86	56	4 $\frac{1}{2}$	96.9
	22	3d	183	208	.88	59	4 $\frac{1}{4}$	97.2
	23	4th	182	212	.86	56	4 $\frac{1}{2}$	97.0
H. L. H.	Jan. 1912 9	Preliminary	211	252	.84	70	..	97.7
	10	1st	215	246	.87	73	..	98.0
	11	2d	202	238	.85	64	5 $\frac{1}{8}$	97.4
	12	3d	209	239	.87	67	..	97.6
	13	4th	195	245	.80	69	..	97.9

tory quotient of H. H. A. (I) on the fourth day and more particularly H. L. H. on the fourth day, is very marked. We are at a loss to explain this fall satisfactorily.

DISCUSSION OF RESULTS.

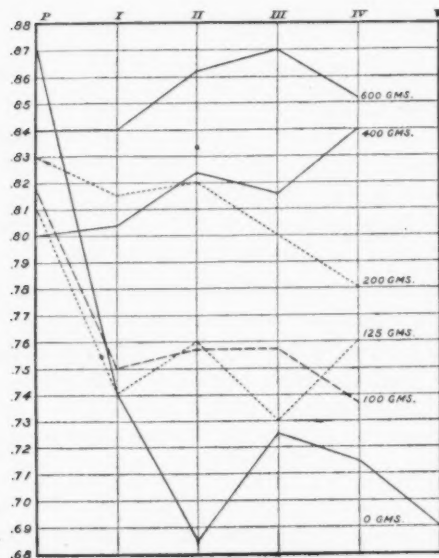
On inspection of these results it will be observed that a given diet with the same individual will generally lead to the same respiratory exchange on the following morning; that is, after a given diet, one finds essentially the same rates of carbon dioxide output and oxygen consumption and consequently the same respiratory quotient. This is very marked in the case of the oxygen consumption, since different diets with the same subject do not necessarily lead to the same consumption of oxygen, even when the protein content and caloric values are the same. In a few cases, especially when using a large amount of carbohydrates, it is necessary to continue the diet for two or even three days before a constant carbon dioxide output, and therefore a constant respiratory quotient, can be obtained.

The observation that a given diet usually produces the same respiratory exchange is of especial value in a study of the effect upon the metabolism of superimposed factors, such as work, food, or stimulants, when a constant and definite base-line is desirable or obligatory. The respiratory exchange and "nüchtern" metabolism, generally after but one day of a diet, will thus give a definite base-line, which will be constant for any one individual and therefore probably proportional for all subjects.

It may be noted that with the same diet different individuals give approximately the same respiratory quotient, especially after one or two days. The only marked exception in our experiments to this general statement is noted in the case of C. B. S. with 400 gm. of carbohydrate (diet No. 5). It would appear, therefore, from these experiments that but little difference exists among normal people, under the same general conditions of life, as to the storage of carbohydrate after a given diet.

From these experiments, also, it is very clear that the respiratory quotient, under "nüchtern" conditions, is high or low according as the diet of the preceding day is high or low in carbohydrate. Furthermore the higher the amount of carbohydrates, the higher is the respira-

atory quotient in practically a direct relation. The chart, which shows the average respiratory quotient obtained each day of the several diets for all subjects, explains this perhaps more clearly. For instance, with a carbohydrate-free diet, the respiratory quotient ranges from



Average respiratory quotients for the preliminary (P) and four to five (I-V) succeeding days with diets containing varying amounts of carbohydrates.

about 0.685 to 0.725; with 100 gm. of carbohydrate and 125 gm. of carbohydrate a day, which are essentially the same, the quotient is from 0.73 to 0.765; 200 gm. of carbohydrate a day produces a quotient of 0.78 to 0.82, 400 gm. of carbohydrate from 0.81 to 0.84, and 600 gm. of carbohydrate from 0.84 to 0.87. With each diet the respiratory quotient ranges between certain definite limits, these ranges being independent of one another except in the first days of the two diets containing 200 gm. of carbohydrate and 400 gm. of carbohydrate re-

spectively. If a larger number of experiments had been made, however, one may safely predict that even this overlapping would have disappeared.

In the case of F. P. R. with diets Nos. 5 and 6, and of H. H. A. (II) with diet No. 6, a gradual rise in the respiratory quotients may be observed on the first days. This points to the possibility of a gradual accumulation of carbohydrate in the body, indicating that the rather low supply of body carbohydrate at the beginning of the diet could not be raised in one day to a level which might be said to be commensurable to the diet. It is also clearly shown that this accumulation will not be continued indefinitely, since the quotient in two of these cases reached a fixed level above which it did not rise on subsequent

days. In no urine with the diet containing 600 gm. of carbohydrate was sugar found in excess of the slight normal reducing power.

The selective capacity of the tissues in choosing the materials which it will metabolize or burn is emphasized by these experiments. That the body can make use of diets containing 100 gm. of carbohydrates and 600 gm. of carbohydrates with almost equal facility confirms the view that carbohydrate is a decidedly labile body constituent. The utilization by the body of 600 gm. of carbohydrate a day without glycosuria is probably explained in most part by the correspondingly increased combustion of carbohydrate indicated by the respiratory quotient the following morning, and in part perhaps by the formation of fat from sugar. This efficiency is of especial interest in view of the fact that the 3000 calories, of which 2400 calories were from carbohydrates alone, probably supplied each subject with a ration more than sufficient for maintenance.

In connection with these diets a constant protein intake was maintained so far as possible, in order to avoid the effect upon the results of a varying protein metabolism. All of the urines were collected, but as the other excreta were not analyzed for nitrogen, and our knowledge of the amount of protein intake is but approximate to 5 gm., we do not feel justified in giving any prominence to the urine data. However, the nitrogen excretion for twenty-four hours with diets Nos. 2 and 3 was consistently higher than with diets Nos. 4, 5, and 6, even sometimes as much as 20 to 30 per cent.

CONCLUSIONS.

1. With the same individual and the same diet on the preceding day, the respiratory exchange in the morning without food will always be essentially the same. When the diet contains considerable carbohydrate, and the supply of body carbohydrate at the beginning of an experiment is small, it may be necessary to continue the diet two or possibly three days before this agreement in the respiratory exchange is obtained.

2. With different normal individuals the same diet will lead to essentially the same respiratory quotient the next morning without food.

3. The supply of body carbohydrate, as shown by the respiratory quotient after digestion has ceased, bears a distinct relation to the quantity of carbohydrate in the preceding diet.

4. A diet of 600 gm. carbohydrate and 3100 calories a day for four days was not sufficient to produce glycosuria in men with sedentary occupations.

STUDIES ON THE PULMONARY CIRCULATION.—I. THE
PRESSURE VARIATIONS IN THE PULMONARY CIR-
CULATION OF THE DOG STUDIED BY A NEW PULSE
PRESSURE INSTRUMENT.

BY CARL J. WIGGERS.

[From the Physiological Laboratory, Cornell University Medical College, New York City.]

PREVIOUS WORK.

THE mean pressure existing in the pulmonary circulation of the dog has been determined in artificial respiration experiments by Beutner, Lichtheim, Openchowski, Bradford and Dean, Velich and Wood.¹ The extremes of mean pressure as well as an average of each observer's results are schematically shown in Fig. 1. It is quite obvious, however, that values thus obtained are not necessarily identical with those occurring in the animal when breathing is natural, for (1) the mechanical influences of artificial respiration are substituted for those incidental to natural breathing, and (2) the degree of artificial respiration produces a variable CO₂ content in the blood which causes the rate and systolic discharge of the heart and possibly also the calibre of the pulmonary vessels to change. Hence artificial respiration modifies the pulmonary arterial pressure. That the averages of such wide variations as different workers obtained happen to correspond with the mean pressure of a single investigator who studied the pulmonary circulation during natural breathing may be regarded as a mere chance rather than, as Tigerstedt¹ implies, evidence that artificial respiration introduces no material error. Only two experimenters, Knoll² in rabbits and Plumier³ in rabbits and dogs,

¹ TIGERSTEDT: *Ergebnisse der Physiologie*, 1907, ii, 2, p. 528.

² KNOLL: *Sitzungsberichte der Akademie der Wissenschaften in Wien*, 1888, xcvi, p. 207.

³ PLUMIER: *Archiv internationale de physiologie*, 1904, i, p. 176.

have investigated the pressure in the pulmonary arteries during natural breathing. Plumier found that, although the mean pressure in dogs ranged very low, there occurred in addition variations of pressure with each respiratory cycle which were often greater than the average mean pressure itself (Fig. 1, *P*). It appears from such observations that the respiratory variations in the pulmonary arterial pressure, so completely disregarded in open chest experiments, become, in many

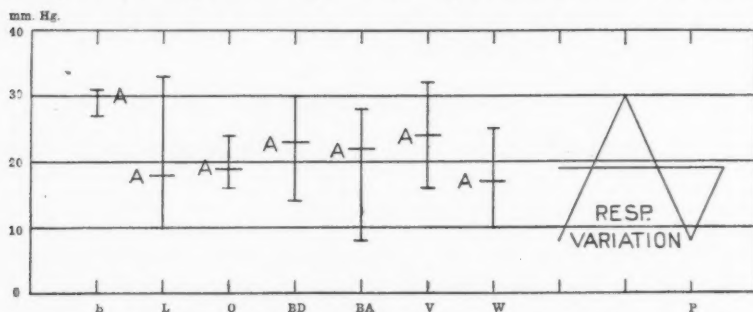


FIGURE 1. — Diagram showing the extremes of mean pressure and their average (*A*), as recorded in dogs by Beutner (*B*), Lichtheim (*L*), Openchowski (*O*), Bradford and Dean (*BD*), Bayet (*BA*), Velich (*V*), Wood (*W*), and Plumier (*P*).

respects, more important for investigation than the mean pressure height itself.

In 1910 the writer⁴ made a report upon the maximal and minimal pressures found in the pulmonary arteries of dogs, as registered by valved mercury manometers. The results upon which this report was based are shown in Table I. They show that in experiments where the mean pressure averaged only 18 mm., the maximal pressure averaged 31.3 mm. and the minimal pressure 5.9 mm.

NATURE OF PRESSURE CHANGES.

Records of the pulmonary arterial pressure with a membrane manometer show that during each cardiac cycle variations of pressure occur, the magnitude of which depends on the rate and systolic discharge of the heart, as well as on the resistance and capacity of the

⁴ WIGGERS: this Journal (Proceedings of Society), 1910, xxvii, p. xxi.

pulmonary vessels at the time of such discharge. The pressure variations during systole and diastole may be designated, as in the systemic circuit,⁵ the *actual pulse pressure*. Records with membrane manometers indicate, however, that neither consecutive systolic and diastolic pressures nor their differences, the pulse pressures, are equal, but vary during each respiratory phase in a manner more marked than in the systemic circuit. For the extreme differences in pressure thus created during several respirations, the term maximal-minimal pressure difference is reserved, since these pressures are the ones recorded by mercury manometers (Fig. 2, *MX-MN*).

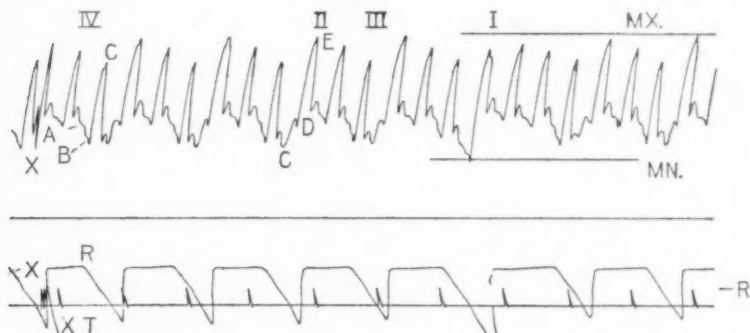


FIGURE 2.—Pressure variations in the pulmonary circuit recorded with a membrane manometer of a vibration rate of 22.3 per second, showing difference between actual pulse pressures (Waves I, II, III, IV) and maximal-minimal pressure difference (*MX-MN*). *X*, relative position of levers. *R*, respiration, downstroke in inspiration. *T*, time in seconds (small letters referred to in text).

Such a simple differentiation between the actual pulse pressure and the maximal-minimal pressure difference presents certain difficulties in the pulmonary circuit, however, which are not met with in the systemic arteries. In only a comparatively small number of animals are the number of heart beats to each respiration so nicely timed that inspiration and expiration fall uniformly during either systole or diastole of the heart. When expiration occurs during systole, it expresses itself as an increased upstroke followed by a correspondingly high diastolic pressure (Fig. 2, Wave I), which offers a support for the succeeding systolic pressure. When, however, expiration occurs

⁵ WIGGERS: *Journal of experimental medicine*, 1912, xvi, p. 174.

TABLE I.

Exp. No.	Systemic mean pressure.	Heart rate.	Pressures with valved manometers.			Pressures
			Max.	Min.	Diff.	Systolic.
432	80	...	27	10	17	..
433	98	100	36	2	34	30
...	104	155	37	4	33	28
...	112	155	36	5	31	31
...	104	158	34	4	30	30
...	100	170	29	5	24	..
...	...	180	29
...	...	180	29	3	26	28
...	...	175	33	2	31	28
...	...	180	30	2	28	27
...	...	170	34	2	32	25
434	92	...	48	7	41	..
...	92	...	43	8	35	38
...	80	38
...	80	...	41	10	30	32
...	70	...	36	10	26	34
...	70	...	38	10	28	33
436	100	...	36	..	36 ?	..
...	100	...	38	1	37	..
...	112	...	41	2	39	..
437	106	210	30
...	116	200	31
438	126	108	36	6	30	36
...	110	144	37	12	25	32
440	100	150	40	15	25	35
Average.			31.3	5.9	25.4	31.1

TABLE I.

by Hürthle manometer. Inspiration.		Pressure by Hürthle manometer. Expiration.			Respiration.	
Diastolic.	Pulse pressure.	Systolic.	Diastolic.	Pulse pressure.	Rate.	Depth.
..	mm. ..
10	20	34	16	18
11	17	31	15	16	18	8
11	20	34	16	18	22	8
10	20	34	16	18	22	6
..
8	21	30	15	15	27	7
10	18	33	17	16	27	8
9	19	35	16	19	33	8
8	19	35	17	16	30	9.5
8	17	35	18	17	36	12
..
18	20	49	28	21	18	11
15	23	43	22	21	24	16
18	14	38	22	16	30	5
18	16	36	23	13	27	5.5
18	15	40	24	16	27	9
..	21	5
..	21	6
..	21	9.5
8	22	24	20	14	27	7
15	16	36	22	14	27	9
20	16	40	23	17	24	5
8	24	35	8	27	..	9
18	17	43	20	23
12.3	18.8	36	17.1	19

during diastole, it causes a small elevation of diastolic pressure which offers a support to the next systolic upstroke (Fig. 2, Wave II). The question then arises whether to regard *C-E* or *D-E* as the true pulse pressure during the next heart beat. If the former is selected, expiration always causes an increased pulse pressure, but if the latter is chosen, it often causes a smaller pulse pressure. When inspiration occurs during systole, no marked effect on the upstroke is apparent (Wave III, Fig. 2). When it falls during diastole, however, it produces a distinct depression. Such cases offer a choice between the variations *A-C* or *B-C* as the true pulse pressure.

The object for which the pressure variations are measured largely determines the choice in these cases. If it were proposed to utilize the actual pulse pressure in the pulmonary circulation as an index of the systolic discharge of the right heart, it would be desirable to disregard the respiratory factor influencing the pressure during diastole as much as possible, and accordingly it would be proper to regard the distance *B-C* of Wave IV and *D-E* of Wave II as true pulse pressures. Inasmuch as it is very questionable whether such actual pulse pressures taken in the pulmonary circulation during natural breathing offer a criterion of the systolic output (it being impossible to estimate the influence of inspiration and expiration when they occur during systole), and, furthermore, as the determination of the extent of both cardiac and respiratory influences was desired, it seemed preferable in this work to regard the difference between any systolic pressure and the lowest part of the diastolic pressure immediately preceding it as the actual pulse pressure change occurring during the interval of a cardiac cycle (*e. g.* the variations *B-C* and *C-E*). To an average of ten of these actual pulse pressures the term "average pulse pressure" is frequently applied in this paper.

APPARATUS EMPLOYED TO REGISTER PULSE PRESSURE.

Before undertaking a quantitative investigation of the pressure variations in the pulmonary circuit, it becomes fundamentally important to satisfy oneself that the instrument employed reproduces the systolic and diastolic pressures without error.

Recognizing the inability of maximal and minimal valved manometers to record actual pulse pressures, the pressures in the pulmonary

artery were simultaneously recorded in a preliminary investigation⁴ with a calibrated membrane manometer. The results showed, however, that the highest and lowest pressures deciphered from the record of a membrane manometer failed to agree with those of the valved manometers (Table I). These discrepancies led to a study of the errors incurred by membrane manometers, which, as the reasons for their existence gradually became clear, resulted in the development of a new instrument which controlled the errors of the ordinary membrane manometer.

Reasons for the inaccuracy of membrane manometers. — The studies of Frank⁶ indicate that the quality of an instrument (*i. e.*, its ability to reproduce pressure changes accurately) may be estimated by determining the vibration period (T), or its reciprocal, the number of vibrations per second. In a simple manometer system (exclusive of registering apparatus) $T = 2\pi\sqrt{\frac{M'}{E'}}$, where M' , the effective mass, depends directly on the specific gravity of the fluid, the length of the manometer column, and, on account of a friction factor, inversely on the cross-section of the manometer tube, and where E' , the volume-elasticity coefficient (ratio of pressure increase to volume displaced), depends, when air bubbles and elastic connections are avoided, entirely on the character of the membrane or spring restraining the fluid within the manometer system.

The quality of an instrument, however, is not the only index of an instrument's usefulness. In order to minimize the risk of error in translating recorded pulse pressures into numerical figures by comparison with a calibration scale, it is necessary that these pressure variations should be reproduced on a feasible scale. In the study of the pulmonary circulation it was desirable that the ratio between the recorded variation and the actual pressure change should not be less than 1:5. As the quality of an instrument increases, however, the excursions of the elastic membrane decrease, hence it becomes necessary to magnify these movements before they are recorded. When such magnification must be obtained by ponderable levers and recorded with friction on smoked surfaces, the reduced mass of the lever (m) (*i. e.*, one third of the lever length, the square of its magni-

⁶ FRANK: *Zeitschrift für Biologie*, 1903, xliv, p. 445; 1908, I, p. 309.

fication (v^2), and its mass per unit area (a)) changes the formula for the vibration period to $T = 2\pi\sqrt{\frac{M'}{E'} + \frac{m}{\eta}}$, a formula in which η designates the deforming action of the lever on the membrane. To judge from the lengthened vibration period, an increase of sensitiveness, whether obtained by decreasing the volume-elasticity coefficient or by increasing the magnification of the membrane movement, decreases its quality.

Studies with his optical manometer led Frank⁷ to the conclusion that an instrument with a vibration rate of at least 40 per second was required in order to register pressure changes in the arterial circulation correctly, but, according to this investigator, the best membrane manometer tested had a vibration rate of 30 per second, while the rate of many instruments in common use range between 12 and 17 per second. The writer has also determined the vibration rate of a number of membrane manometers available in the laboratory. The best of these showed under the most favorable conditions, and when the sensitiveness did not exceed 1:10, a rate of 22 to 28 per second, but when the sensitiveness was increased to the desired ratio of 1:5, the vibration rate dropped to one half of this figure. According to this criterion, then, the ordinary membrane manometer is not capable of registering the pressure variations correctly.

The chief faults of these instruments consist in the fact that, due to the momentum acquired by the movable parts, the writing point is carried too far in its rise. To counteract this effect of great mass inertia, artificial resistance is introduced, in practice, by means of a damping cock. This, however, tends to produce so marked a deformation of the record in a direction opposite to the effect of mass inertia that the matter is overdone unless we possess some criterion by which the correct amount of damping may be judged. Hürthle⁸ pointed out that the damping should never be so great that the maximal variations which it is expected to record cannot be completed in the time interval available for such changes in the animal, and has suggested the simple expedient of damping until the contour of the damped curve does not deviate from that of an undamped manometer. Porter⁹

⁷ FRANK: *Zeitschrift für Biologie*, 1905, xlv, p. 512.

⁸ HÜRTHLE: *Archiv für die gesammte Physiologie*, 1890, xlvii, p. 8; 1910, cxxxvii, p. 145.

⁹ PORTER: *Journal of physiology*, 1892, xiii, p. 513.

gauged the optimum degree of damping by comparing the recorded pressure extremes with maximal and minimal valved manometers.

With these standards as to the proper amount of damping, it is commonly believed to be quite or entirely a matter of technic to obtain records which are true as regards their amplitude. Such, however, has not been the experience of the writer. As before pointed out, the highest and lowest pressures recorded by a membrane manometer failed to agree with those given by maximal and minimal valved manometers. The chief deviation lay in too high a diastolic pressure. It was furthermore found that, in the few instances where by suitable damping it was possible to make both the highest systolic and lowest diastolic pressures agree reasonably well with the maximal and minimal pressures of valved manometers at the beginning of an experiment, this correspondence did not hold good to the end. The factors which account for such variations are difficult to determine in each case. They are probably variable and numerous. Among those probably entering may be mentioned (*a*) an unequal stretching of the rubber membrane, (*b*) a variable friction in the axes and between the drum surface and writing point, (*c*) a change in the force of the heart to which the damping was originally adapted, (*d*) an accidental and unavoidable change of artificial resistance (*e. g.* by lodgment of particles of vaseline in the stopcock), and (*e*) a modification in the natural resistance within the manometer system due to the entrance of blood into the manometer tubes or to the formation of a coagulum in the cannula.

It seems, therefore, that if instruments with low vibration rates, writing with ponderable levers on smoked surfaces, are to approach quantitative accuracy, it is necessary to standardize them frequently against maximal and minimal valves. In doing this, however, so many instances were found in which it was impossible to adjust the damping so that both systolic and diastolic pressures corresponded at the same time with that read in valved manometers, that a number of procedures were devised to overcome this difficulty. This led eventually to the construction of a sensitive pulse pressure instrument which, being capable of rapid and repeated standardization, gives correctly the extremes of pressure during the interval of any cardiac cycle. It may be added, parenthetically, that no claim is made as to the instrument's ability to depict correctly the contour of pressure waves.

Description of instrument.¹⁰—The pulse pressure instrument (Fig. 3) consists essentially of the two registering manometers, *S* and *D*, connecting by stopcocks 1 and 2 with the valve chamber *C*.

Each registering manometer consists of a capsule (*A*) having an opening 7 mm. in diameter. Both capsules are covered with a membrane under such a degree of tension as to give a resistant sensation to the touch. The membrane covering the capsule *D* is chosen slightly lighter than that covering capsule *S*. The lever system is supported on a framework (*B*) which is set over the manometer capsule (*A*), and by rotating about it as an axis, enables the levers to write at

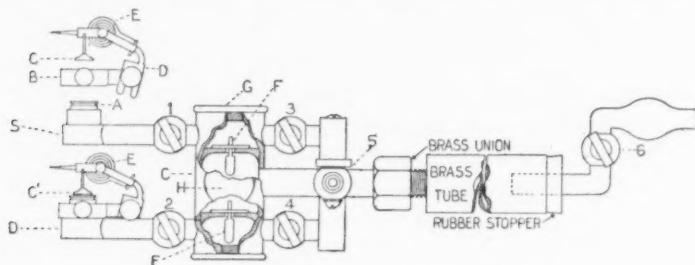


FIGURE 3. — Pulse pressure instrument (letters referred to in text).

any angle to the drum and permits an independent delicate adjustment of each lever to the smoked surface. This rotatory adjustment is made possible by the fact that the aluminum disc (*C*) cemented to the rubber membrane does not partake of the rotation, since its movement is transmitted to the lever through a ball and socket joint. The hollow straw lever (not shown in Fig. 3), together with its paper pointer, measures 10 cm., weighs 30 mg., and magnifies 25 times. The lever can be raised and lowered by a slotted bar (*D*), supporting the lever axis. In the case of the upper manometer its weight is further supported by a hair spring (*E*) to insure a prompt *upward* movement, whereas the lower lever is equipped with a similar spring (*E'*) exerting a *downward* pull.

The valve chamber (*C*) is divided by two dome-shaped valve seats into three compartments. The upper and lower compartments both communicate through a stopcock with the registering manometers,

¹⁰ I am obliged to Mr. G. F. Soderstrom for a sketch of the apparatus.

and, if stopcocks 3 and 4 are open, also with the tubes connected to the artery. If these cocks are closed, however, they communicate with this tube (*T*) only through the middle compartment. The floor of the upper chamber is guarded by a maximal valve (*F*) which may be reached by removing screwcap (*G*), and the top of the lower chamber is covered by a similar minimal valve (*F*) which becomes accessible through the lateral screwcap (*H*). The design of the valves is shown in Fig. 3. They are made of brass and weigh exactly .9 gm. Firm closure is obtained by a small circlet of rubber dam applied to the lower surface of the horizontal portion of each valve. The weight of the valves is important. They should be sufficiently heavy so that

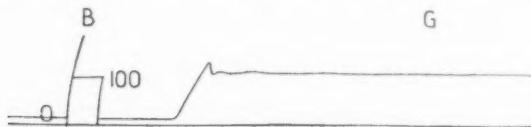


FIGURE 4. — Curves showing that when pressure change is too rapid in proportion to weight of valves, the maximal pressure is recorded higher than actually existing. Pressure variations in all tests, 0–100 mm. *B*, pressure directly recorded. *G*, pressure recorded through valves.

the maximal or minimal pressure is not recorded through them during the interval of a single pulse beat. Otherwise there is a tendency in sensitive instruments for the lever to record the maximal and minimal pressure lines respectively higher and lower than they should (Fig. 4). On the other hand, they should not be so heavy as to interfere with the full pressure record within the time interval occupied by 5 or 6 heart beats. That these conditions are complied with in the case of valves weighing .9 gm. is shown in that the maximal pressure, when recorded through the valves, is not immediately recorded to its fullest height, but after a time interval of approximately .12 second (Fig. 5).

Stopcocks 1 and 2 are utilized to dampen the manometers (damping cocks), while 3 and 4 by their closure shunt the pressure through the middle chamber and the maximal and minimal valves (standardization cocks). Stopcock 5 connecting by tubing with a pressure flask and a mercury manometer similar to that described by Guenther and Lombard¹¹ serves for calibrating and filling. The brass communication tube is 25 cm. in length and 1.8 cm. in internal diameter. The

¹¹ GUENTHER and LOMBARD: this Journal, 1910, iv, p. iii.

end communicating with the cannula is closed by a perforated stopper through which an Γ -shaped tube with a stopcock is passed. This permits an adjustment to the level of the cannula by its rotation through cork.

Calibration, standardization, and operation.—The instrument is calibrated in the customary manner by attaching a manometer and pressure bottle to stopcock 5.

After connection with an artery the instrument is operated as follows: Stopcocks 3 and 4 are closed, permitting a registration of the

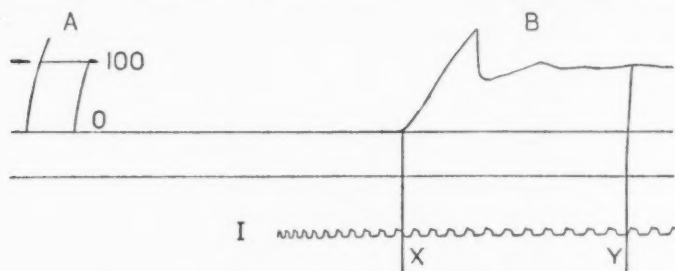


FIGURE 5. — *A* shows 100 mm. level. *B* shows that the interval (*X-Y*) required before maximal pressure is recorded through valves, .9 gm. in weight, is approximately 0.11 second. *I*, time in 1/100 second.

maximal and minimal pressure level by the two manometers. This should require several pulse beats, but is usually carried on while the drum remains stationary. The drum is then allowed to run and trace these levels as a straight line with which, if desired, two ordinate markers can be made to coincide. By adjusting the damping of stopcocks 1 and 2, the writing points are so adjusted that, upon opening stopcocks 3 and 4, in the case of the upper lever, the highest systolic pressure occurring during a respiratory phase coincides exactly with the maximum pressure line, while in the case of the lower lever the lowest diastolic pressure corresponds with the minimal line. This procedure, designated as the standardization, is frequently repeated during the course of an experiment. Fig. 6 shows instances where this standardization was applied in studies of pressures in the pulmonary arteries and the carotid respectively. By applying the calibration scale to the shellacked record, the actual systolic and diastolic pressures during any heart beat can be calculated.

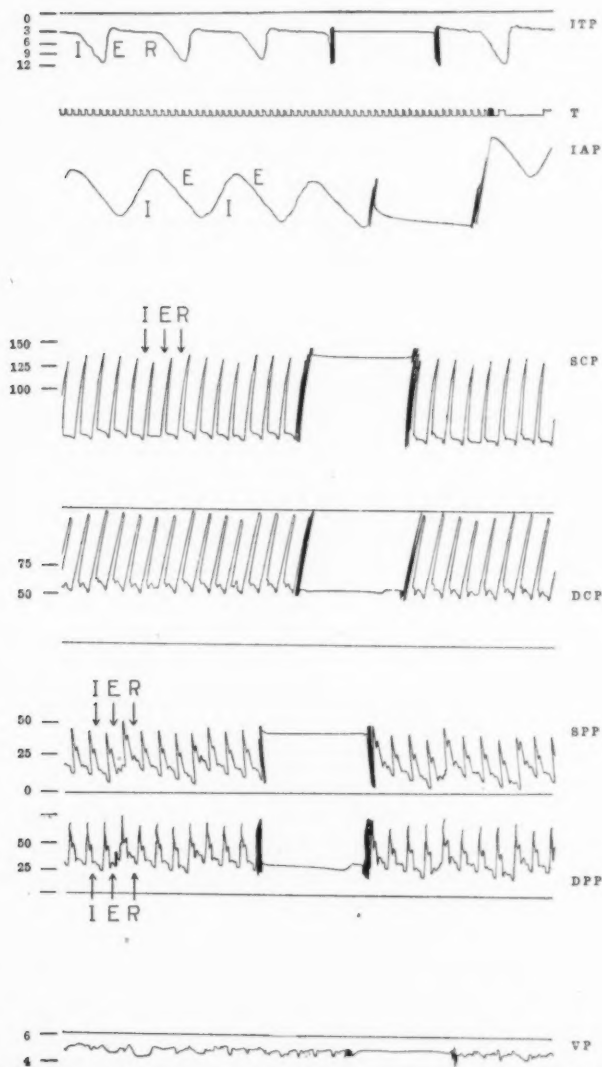


FIGURE 6.—Records showing nature of tracings taken in experiments. *ITP*, intrathoracic pressure changes; *IAP*, intra-abdominal pressure; *SCP*, systolic carotid pressure; *DCP*, diastolic carotid pressure; *SPP*, systolic pulmonary arterial pressure; *DPP*, diastolic pulmonary arterial pressure; *VP*, superior vena cava pressure; *T*, time in 1-5 seconds; *I*, inspiration; *E*, expiration; *R*, expiratory rest.

Test of instrument.—Inasmuch as the principle of this instrument assumes that the highest and lowest pressures registered through its maximal and minimal valves represent the true extremes of pressure in the animal, the question whether the position of rest assumed by the manometer levers depicts the extreme pressures in the vessels

TABLE II.

Carotid pressures recorded by valved mercury manometers.			Carotid pressures recorded by pulse pressure instrument.	
Exp.	Maximal.	Minimal.	Maximal.	Minimal.
371	160	57	157	58
372	148	66	148	68
373	170	64	168	64
441	158	70	160	72
443	126	61	126	60
445	145	36	140	40
447	154	62	154	60
448	250	54	246	56
449	148	63	148	64

demands investigation. In the case of mercury manometers, the mercurial mass is so great that they reach the maximal and minimal levels only after several heart beats. The mass of the membrane manometers, however, is so reactive that the maximal pressures might be quickly reached, and with the greater displacement of fluid in sensitive manometers might register a pressure higher or lower than the extremes of pressure really existing in the animal. Physical tests showed that, while this is possible during a very sudden application of pressure (Fig. 4), it can be relied upon not to occur when the extreme pressures are not recorded during a single pressure change. In the dog, where the greatest changes of pressure take place, it was found, with only a few exceptions, that the minimal and maximal pressures were only attained after several heart beats, and in all of these exceptions this could readily be attained by partly closing stopcock 6. A practical

test of this point was, however, also made by actual comparison of these maximal and minimal pressures of the systemic circuit with those obtained with valved mercury monometers. The results of such comparison, as shown in Table II, show a deviation so small as to be regarded negligible in comparisons of this kind.

OPERATIVE PROCEDURES.

Dogs were anesthetized with chloretone alone, in which case the heart rate was perfectly regular, inspiratory and expiratory variations in cardiac rhythm being eliminated without vagus section.

A record of arterial and venous pressure was taken in all experiments. A long cannula filled with anticoagulation fluid was introduced into the superior vena cava through the right external jugular and connected with a membrane manometer through air transmission, a form of registration at once convenient and, according to Frank,¹² more reliable than liquid transmission in the case of venous pressures.

The following technical precautions were employed in order to obtain pulse pressure tracings from the pulmonary artery under conditions of respiration as nearly normal as possible. A thoracic trocar connected by an air-tight system with a recording tambour was first inserted through an intercostal space and the negative pressure during inspiration and expiration recorded for a short time interval. Under artificial respiration the fifth to the eighth ribs were resected on the right side so that the lower lobe could be withdrawn by gentle traction and the arterial branch to the small lobule accessory to this lower lobe could be dissected from the lung tissue. Ligation of this vessel cut off so small a part of the entire pulmonary circuit that its closure by a clamp even during natural breathing was without any influence on recorded arterial pulse pressures. After applying a clamp which could subsequently be operated by a screw projecting from the repaired chest, a short-necked waxed cannula was securely tied into this vessel and a rubber tube connection was pulled through an intercostal space in such a way that, with the lung reduced, a straight line was formed with the arterial branch. This proved exceedingly important, for if the least tendency to a kinking of the pulmonary artery into which the

¹² FRANK: *Zeitschrift für Biologie*, 1911, lvii, p. 176.

cannula was inserted existed, it became increased when the ribs were raised in inspiration. If this occurred, the lumen of the vessel was reduced during inspiration and the true pressure variations occurring in the large pulmonary arteries were not accurately reproduced. To prevent the elevation of the ribs compressing the rubber tube during inspiration and so introducing an artificial pressure change, a waxed glass tube, 8 mm. in internal diameter, was slipped into the rubber connection until it approximated the cannula. This tube was similarly connected to the pressure-recording apparatus. The thoracic wall was then surgically repaired and rendered air-tight. After stoppage of artificial respiration, which had just previously been increased so that the animal was in apnea, a negative pressure equal to that existing during expiratory quiet previous to operation was re-established by gentle suction through a T-tube inserted into the trocar-tambour system. When natural respiration returned, the rate and depth deviated appreciably from that before operation in only 12 per cent of the experiments. These, of course, could not be regarded as normal and are not included in this report.

QUANTITATIVE STUDIES OF PRESSURE CHANGES.

a. Pressure variations in naturally breathing dogs.—The pressures as recorded in a typical experiment are shown in the tracing reproduced as Fig. 6. Such tracings show, when the negative pressure changes are considered, that each respiratory cycle in the dog may be divided into active inspiration, passive expiration, and an interval of respiratory quiet. Taking the pressures during respiratory quiet as a standard, it is evident that the systolic and diastolic pressures both fall during inspiration and rise during expiration. The extremes of systolic and diastolic pressures as quantitatively estimated during the periods of inspiration and expiration in different experiments are shown in Table III.

The following facts may be established from these figures:

1. The systolic pressure varied 2 to 35 mm. during the respiratory cycles. The average variation was 12 mm.
2. The diastolic pressure varied 2 to 18 mm. during the respiratory cycles, an average variation of 8 mm.

TABLE III.

Experiment.	Heart rate.	Respiration rate.	Intra-thoracic pressure range, mm. of H ₂ O.	Heart respiration ratio.	Pulmonary arterial pressures.					
					Systolic.			Diastolic.		
					Insp.	Exp.	Average.	Insp.	Exp.	Average.
467	96	17	30	1:5.5	45	28.3	35	13.2	6	9.5
470	100	37.5	36	...	23	21	...
...	68	32	30	...	16	12	...
471	180	27	18	1:6.3	38	26	29.4	14	6	10.9
472	150	23	21	1:6.5	55	20	38.2	18	10	13.6
473	150	30	21	1:5	32	30	30.6	18	10	12
474	150	40	30	1:3.2	40	30	36.2	20	10	14.8
482	125	25	10	1:5	45	40	43	31.5	21	27.3
482B	125	40	20	1:3	50	36	40.8	27	12.5	20.2
482C	125	10	40	1:12.5	60	45	50	30	12	22
485	144	45	18	1:3	37	26	32	22	16	12
486	120	40	10	1:3	40	26	33	18	14	15
487	140	17	18	1:8	52	43	48	9	6	8

3. The maximal pressure in different experiments ranged between 60 and 32 mm. The average maximal pressure was 43.3 mm.

4. The minimal pressure in different animals ranged between 6 and 21 mm. and gave an average of 11.9 mm.

5. The greatest *actual pulse pressures* (*i. e.*, highest systolic minus highest diastolic) in different animals ranged between 14.5 and 43 mm., an average of 24.3 mm.

6. The smallest actual pulse pressures (*i. e.*, the lowest systolic minus lowest diastolic) in different animals, ranged from 10 to 23.5, an average of 20.6 mm.

7. The maximal-minimal pressure difference (highest systolic minus lowest diastolic) ranged from 20 to 48 mm., an average of 33.1 mm.

b. The influence of heart rate on pulmonary arterial pressures. — The influence that the duration of the heart cycle exerts on pulmonary arterial pressures can only be determined when the influence of respiration is in abeyance. Such a condition was, as a rule, established by inducing a temporary apnea vagi for an interval of 30 seconds and, in a few instances, which served as corroborative checks, by creating a condition of apnea vera. To avoid serious objection, the over-ventilation should not be so great that the apnea following the discontinuance exceeds 45 seconds. In most of the experiments recorded in this paper, it was much less. The experiments in which the relation of the cardiac cycle to pulse pressures was studied under the above conditions may be conveniently classed in groups according to the nature of the heart rate studies.

GROUP I. — *Experiments showing the effect of different heart rates occurring in different animals during temporary apnea.* In six out of nine of these, the heart rate ranged naturally between 84 and 180. To obtain a comparison of pressures with a wider range, three more were added in which the heart was slowed by vagus stimulation. The procedure in each experiment consisted of the following steps: (1) Stimulation of the central end of the left vagus with a current just sufficient to induce apnea. (2) Recording the maximal and minimal pressures through valves of the pulse pressure instrument in order to establish true maximal and minimal levels. (3) Recording 15 or 20 cardiac pulsations with the manometers damped so that the systolic pressure of one and the diastolic pressure of the other corresponded

with the maximal and minimal line. During apnea the systolic and diastolic pressures were, of course, uniform. The values thus obtained are shown in Table IV.

TABLE IV.

Exp.	Heart rate.	Diastolic pressure.	Systolic pressure.	Pulse pressure.
	per min.	mm.	mm.	mm.
486	25	6	54	48 ¹
486	50	8	40	32 ¹
483	70	10	36	26 ¹
479	84	10	33	23
467	96	10	32	22
470	100	14	31	17
482	125	28	42	14
471	150	15	38	23
472	180	31	42	11
¹ Vagus stimulation.				

Deductions. — The results show that the diastolic pressure is higher in those experiments in which the heart rate is most rapid. The systolic pressure, however, does not show a corresponding change. In fact, between certain ranges of heart rate (*i. e.*, 25-100), it tends to decrease slightly as the heart rate increases.

GROUP II. — *Experiments showing the effect of different heart rates in the same animal during temporary apnea.* — As the detailed conditions in different animals cannot be controlled sufficiently to negative the criticism of a chance variation, it was necessary to corroborate the results by studying the influences of heart rate variation at different times in the same animal. After induction of temporary apnea by stimulating the central end of the vagus, the peripheral end was simultaneously excited. The strength of stimulus was then altered during different periods of apnea and various heart rates thus produced. During each such apnea period the instrument was standardized to

make certain that its damping was properly adjusted to the altered force of the heart which such a change in rate entailed. Owing to the

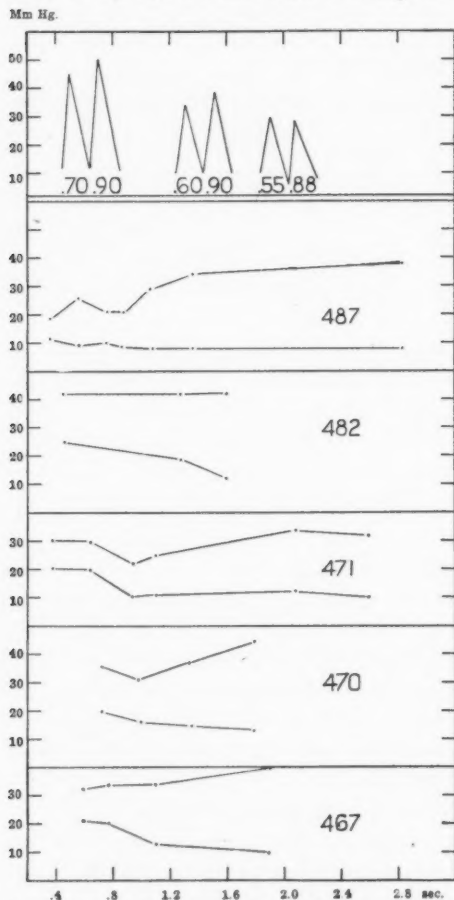


FIGURE 7. — Plots showing the influence of the duration of cardiac cycle on pulmonary systolic and diastolic pressures during apnea. Figures along ordinates represent pressure in millimetres of mercury; those along abscissæ represent duration of heart cycle. Dots indicate precise heart cycles at which observations were made.

tendency of the heart, especially with the stronger current, to escape from inhibition and to thus introduce an irregular rhythm, the variety of heart rates yielded by a single experiment was not so great as might have been desired. The data from different experiments are arranged according to the duration of the cardiac cycles in Fig. 7. They show that as the heart cycle increases in length up to .8 second (approximately) the systolic pressure either undergoes no change or displays a slight tendency to decrease. When the heart cycle becomes longer than 1 second, however, the systolic pressure begins to exceed that of faster heart rates.

This tendency of the pulmonary systolic pressure to deviate in direction from the diastolic in the case of slow beats is not shown in the systemic circuit and requires a few words of interpretation.

Since any decrease in the duration of the cardiac cycle occurs largely at the expense of

diastole, the diastolic pressure in the systemic circuit increases *pari passu* with the heart rate, obviously because the interval during which blood can leave the arterioles is decreased. The resistance in the pulmonary circuit is so low, however, that a pronounced reduction of the diastolic interval is necessary before the flow of blood from the vessels is materially impeded. This probably explains why the diastolic pressure is so constant during variations of heart rate as wide as 200 and 80.

Were the systolic output of the right ventricle at all heart rates equal, we should anticipate a corresponding effect on the systolic pressure. The cardiometer studies of Henderson¹³ have shown, however, that every increase in heart rate above 60 per minute is accompanied by a decreased output. As it is found that the marked increase in systolic pressure occurs when the heart rate is less than 80, it is a reasonable inference that the high systolic pressure at slow heart rates is probably an expression of the overbalancing influence of the greater systolic output. When the heart slows down, we have, in both the systemic and pulmonary circuits, two forces tending to modify the systolic pressure, the greater output tending to increase the systolic discharge, the prolonged diastole tending to reduce it by lowering the diastolic support. In the pulmonary circulation the diastolic pressure is always so low, however, that the latter factor is minimal.

Application of results. — It is well known that the hearts of many animals undergo a rhythmical acceleration during each inspiration and a retardation during each expiration. When, as is often the case of the dog, these variations become extreme, they may exert so marked an influence on systolic and diastolic pressures that the cardiac changes overbalance the mechanical influences of respiration and determine the trend of pressure variations (Plumier³). In man, however, these variations are not extreme, and the heart cycle rarely varies beyond the range .60 to .90 sec. (Lombard, Pillsbury¹⁴). The studies on the influence of heart rate show that it is precisely within this range that the variation in the length of the cardiac cycle is practically without influence on the diastolic pressure and that its influence on the systolic pressure is very slight. This is shown in Fig. 7, A, where the actual pulse pressures during heart cycles between these ranges are compared

¹³ HENDERSON: this Journal, 1908, xxiii, p. 345.

¹⁴ LOMBARD and PILLSBURY: this Journal, 1899, iii, p. 201.

in three experiments. It becomes, therefore, very questionable whether the secondary respiratory rhythm found in man is able to influence the pulmonary arterial pressure to any mentionable extent.

c. Influence of respiration on pulmonary arterial pressures.—The total mechanical influence exerted by respiratory movements on the pulmonary arterial pressure may be determined by comparing the average pulse pressure and the maximal-minimal pressure difference during normal breathing and temporary apnea vagi. The influence of inspiration and expiration may be separately estimated by comparing the actual pulse pressure during inspiration and expiration with that

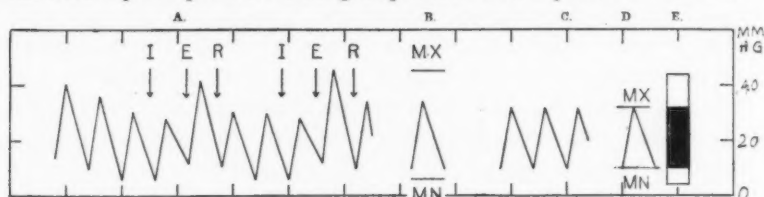


FIGURE 8.—Plot of data from Experiment 467: *A*, actual pulse pressures, natural breathing; *B*, average pulse pressure and maximal-minimal pressure difference during normal breathing; *C*, *D*, same in apnea; *E*, maximal and minimal pressures during breathing and apnea compared. Cardiac cycles (.63 second) each represented by 35 paces on abscisse.

occurring during the interval of respiratory quiet. These relations were worked out in three cases. A single experiment may be quoted.

Experiment 467.—November 27, 1911. Dog, weight 21 kg. Heart, regular; rate, 96 per minute; respiration rate, 17; intrathoracic pressure variation, -2 to -4.5 mm. Hg. Respiratory cycles, divided into active inspiration, passive expiration, and respiratory rest, have a ratio 2:1:3—heart respiration ratio was $1:5\frac{1}{2}$. The results are shown in *A*, Fig. 8. The systolic pressure ranged from 28.3 mm. in inspiration to 45 during expiration, while the diastolic pressure during these respiratory phases varied from 6 to 13.2 mm. Taking the pressure during the interval of respiratory quiet as a standard, it becomes evident that both systolic and diastolic pressures decrease during inspiration and increase during expiration; but the relative changes of systolic and diastolic pressures are such that the pulse pressure is greatest during expiration and least during inspiration. The maximal-minimal pressure difference of 39 mm. contrasts with the greatest pulse pressure of 32 mm. and the average pulse pressure of 25.58 mm. (*B*, Fig. 8).

After the production of apnea vagi for a short interval of time, the systolic pressure (C, Fig. 8) was uniformly 32 mm. and the diastolic 10 mm., giving an actual pulse pressure of 22 mm., which now became synonymous also with the average pulse pressure and the maximal-minimal pressure difference. If the maximal-minimal pressure differences during apnea and normal breathing are now compared, it becomes evident (Fig. 8, E), that the difference between 45 mm. and 32 mm. in the case of the maximal pressures and between 10 mm. and 6 mm. in the case of the minimal pressures is contributed by respiratory movements. Expressed in percentile figures, ordinary respiration contributes 40 per cent to the height of the maximal and 14 per cent to the depth of the minimal pressure. In the two other experiments comprising this group, respiratory movements contributed 38 per cent and 32 per cent respectively to the maximal and 10 per cent and 25 per cent to the minimal pressures.

Another group of six experiments were studied with reference to the respective influence of rate and depth of respiration on pulmonary arterial pressures. A typical case of this group may also be considered in detail. In Experiment 482, in which the heart rate was uniformly 125 per minute, the respiration during three periods was as follows: I. Rate, 25; depth, 5 mm. II. Rate, 40; depth, 8 mm. III. Rate, 10; depth, 20 mm. During these periods the following results were obtained:

Period.	Maximal pressure.			Minimal pressure.		
	Breathing.	Apnea.	Percentage pressor action.	Breathing.	Apnea.	Percentage depressor action.
I	mm. 50	mm. 42.5	17	mm. 21	mm. 28	25
II	50	42.5	17	12.5	28	44
III	60	49.4	46	12	24.8	52

These figures, corroborated by the other experiments, indicate that depth of respiration plays the major part in determining the extreme pressures within the pulmonary circuit. In fact, as long as the heart rate remains unchanged, it is difficult to picture how an increase in

respiratory rate could, in any way, influence the pulmonary arterial pressure. Any increase in respiratory rate occurs, as a rule, at the expense of the expiratory pause and not in the duration of inspiration or expiration. It is only when respiration becomes so rapid that both

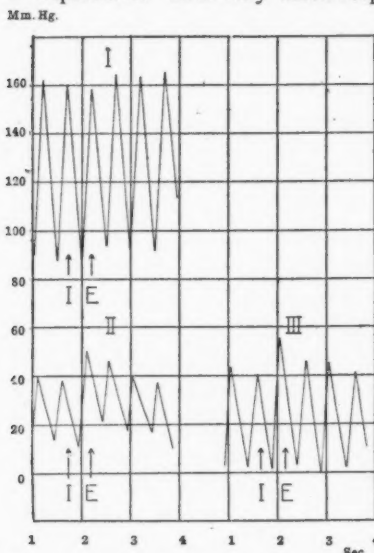


FIGURE 9.—Three plots from Experiment 486, showing relations of carotid, pulmonary arterial, and right ventricular pressures (cardiac cycle, .50 second).

its variations during inspiration and expiration precisely. The expiratory increase in systemic pressures is, however, delayed for the interval of one heart beat.

inspiration and expiration fall during the same cardiac systole or diastole, that the natural relation can be disturbed.

Relation between pulmonary, arterial, right ventricular, and carotid pressures.

—Inasmuch as only two pulse pressure instruments were available, it was necessary to record at separate but closely following intervals, first, the pulmonary arterial with the carotid pressure, and, secondly, the pulmonary arterial with the right ventricular pressure.

Fig. 9 shows the relations thus established. It becomes interesting in such a comparison of pressures to note that the intraventricular pressure exceeds the pulmonary systolic pressure by only a few millimetres in corresponding heart beats and follows

SUMMARY.

By measuring the pressures in the pulmonary artery of naturally breathing dogs with sensitive membrane manometers, the damping of which was standardized against maximal and minimal valves, the following pressure relations were established:

1. The maximal pressure averaged 43.5 mm., the minimal 11.9 mm.
2. The actual pulse pressure was smallest during inspiration, averaging 19.8 mm., and greatest during early expiration, averaging

23.3 mm. In every case it was considerably smaller than the maximal-minimal pressure difference, which averaged 31.4 mm.

3. The length of the cardiac cycle modified the pressures in the following manner: An increase in the heart cycle caused a slight and graded reduction of diastolic pressure. A lengthening of the cardiac cycle from .3 to .8 second (approximately) caused a tendency to decrease the systolic pressure. Further reduction inaugurated an increase in pressure.

4. Variations in the length of the cardiac cycle such as occur in man (extremes, .60 to .90 second) produce no very appreciable influence on the systolic and diastolic pressures.

5. The mechanical action of respiratory movements contributed approximately 32 to 40 per cent to the height of the maximal, and 10 to 25 per cent to the depth of the minimal, pressures to which the vessels are exposed. The depth of respiration and the corresponding variation in intrathoracic pressure determine the extent of this contribution.

6. The systolic pressure in the pulmonary arteries is exceeded only a few millimetres by the systolic pressure within the right ventricle, the respiratory variations of which correspond to those in the pulmonary arteries.

A NUTRITION INVESTIGATION ON THE INSOLUBLE CARBOHYDRATES OR MARC OF THE APPLE.

By EDWARD C. SCHNEIDER.

[From the Department of Biology of Colorado College, Colorado Springs, Colorado.]

IN recent years that group of carbohydrates called hemicelluloses,¹ which is distinguished from cellulose on the one hand and the majority of soluble polysaccharides on the other by being capable of hydrolysis on boiling with dilute mineral acids and resistant to the action of diastase, has been the subject of numerous investigations. In this group are the pectins,² to which has been ascribed the gelatinizing property of many fruit and other plant juices. They are widely distributed among plants, but are not reported to have been found in animal tissue. Many botanists³ consider that pectic compounds are always associated with cellulose in the cell wall, while some hold that the intercellular substance or middle lamella is composed of a calcium salt of pectic acid. These compounds are known as pectoses, and from them on boiling with water the pectin is obtained. Czapek⁴ decides that "it is uncertain whether they form a definite class of cell wall substances." Bigelow, Gore, and Howard⁵ suggest that all insoluble pectin bodies occurring in the vegetable world are really in combination with cellulose.

The pectins occur both in the juice and the marc of fruits and vegetables, and have been the subject of many chemical investiga-

¹ For a good review of the literature on these, see SWARTZ: Transactions of the Connecticut Academy of Arts and Sciences, 1911, xvi, pp. 247-382.

² For literature, see BIGELOW, GORE, and HOWARD: United States Department of Agriculture, Bulletin of the Bureau of Chemistry, 1905, No. 94, pp. 67-89.

³ See GREEN's Vegetable Physiology, pp. 41-46; also the numerous papers of MANGIN: Comptes rendus, cix, p. 579, cx, p. 295, cxvi, p. 653.

⁴ CZAPEK: Biochemie der Pflanzen, i, p. 545.

⁵ BIGELOW, GORE, and HOWARD: *Loc. cit.*

tions. Scheibler,⁶ in 1868, secured from the marc of beets a crystallizable sugar which he called arabinose. Later Weisberg⁷ confirmed the opinion of Scheibler by obtaining a furfural yielding sugar from the pectin of beet marc on hydrolysis with sulphuric acid. In 1889 Wohl and Van Niessen⁸ showed the presence of 14.6 per cent of galactan in the beet marc. Next Herzfeld⁹ found the pectins to be combinations of the two hemicelluloses, pentosans, and galactans. "Chemically pectin bodies are characterized by yielding reducing sugars, furfural, and mucic acid in widely varying amounts, according to the source of the pectins and the method employed in isolating them."¹⁰

THE COMPOSITION OF THE APPLE AND ITS MARC.

The average water content of the whole apple varies from about 80 to 86 per cent of the total weight, and the dry matter from about 14 to 20 per cent.¹¹ The marc, which is the portion insoluble in water, includes from 2 to 2.7 per cent of the apple. Bigelow, Gore, and Howard¹² analyzed the air-dried marc from Rhode Island Greening apples with the following results:

Water	14.04 per cent.
Cellulose	33.82 " "
" Starch" (reducing sugars, calculated as starch, obtained by hydrolyzing with weak hydrochloric acid).	27.68 " "
Pentosans	22.79 " "
Protein	3.50 " "
Ash96 " "

They, furthermore, removed the pectin from the marc by boiling in water and secured after six hours a total of 40.84 per cent. The

⁶ SCHEIBLER: *Berichte der deutschen chemischen Gesellschaft*, 1868, i, p. 58.

⁷ BIGELOW, GORE, and HOWARD: *Loc. cit.*

⁸ BIGELOW, GORE, and HOWARD: *Loc. cit.*

⁹ HERZFELD: *Chemisches Centralblatt*, 1891, ii, p. 618.

¹⁰ BIGELOW, GORE, and HOWARD: *Loc. cit.*

¹¹ ALWOOD and DAVIDSON: United States Department of Agriculture, Bulletin of the Bureau of Chemistry, 1904, No. 88, p. 10.

¹² BIGELOW, GORE, and HOWARD: *Loc. cit.*

pectin removed during the first hour yielded 46.58 per cent of pentosan and 54.6 per cent "starch,"¹³ while the pectin removed during the second hour gave 41.46 per cent of pentosan and 30.02 per cent of "starch." They made no mucic acid determinations for galactans.

Marc and pectin from Ben Davis apple.—For the studies recorded in this paper a marc was prepared from Ben Davis apples by the method of Bigelow, Gore, and Howard. After the removal of cores and bruised places the apples were ground to a pulp which was then washed with water until free from sugar. This residue was dried on a radiator, ground to a powder, extracted with alcohol, and then with ether in a Soxhlet's extractor.

The method used for the quantitative estimation of the hemicellulose content consisted in boiling 3 gm. of air-dry marc with 100 c.c. of 2 per cent hydrochloric acid for four hours. The reducing sugar was determined in the neutral filtrate by Allihn's method. The pentosan content was determined by the phloroglucid method,¹⁴ and the galactan content calculated from the yield of mucic acid.¹⁵ One preparation of this marc contained:

Reducing sugars, estimate as dextrose, after boiling . . .	28.00 per cent.
Pentosans	28.02 " "
Galactans	11.57 " "

A pectin was prepared from this marc by hydrolyzing it for several days, at 38° C., with 0.4 per cent hydrochloric acid. It was separated from the filtrate as a colorless, transparent, jelly-like precipitate by adding alcohol. The pectin secured was soluble in water. It was redissolved and again precipitated with alcohol and then spread in thin layers and allowed to dry spontaneously. This pectin was analyzed with the following results:

Reducing sugar, as dextrose, after acid hydrolyses . . .	57.45 per cent.
Pentosans	35.87 " "
Galactans	45.83 " "

¹³ Not starch, but reducing sugars obtained by hydrolysis with hydrochloric acid, calculated as starch.

¹⁴ For phloroglucid method, see Methods of analysis, United States Department of Agriculture, Bulletin of the Bureau of Chemistry, 1908, No. 107, p. 54.

¹⁵ Provisional method for galactan, Bulletin of Bureau of Chemistry, No. 107, p. 55.

A small amount of marc was also prepared from Colorado Greening apples, and from it the pectin was separated by boiling with water for two and a half hours under a return condenser. This preparation was found to contain:

Pentosans	38.89 per cent.
Galactans	49.60 " "

It will be observed that the two pectins prepared by different methods from two kinds of apples, while not of the same composition, are not strikingly different.

BACTERIOLOGICAL STUDIES.

Winogradsky¹⁶ and Behrens¹⁷ have shown that certain bacteria, apparently the butyric acid bacteria, are capable of fermenting pectins. Slowtsoff¹⁸ observed that the xylan in a putrefying mixture disappeared in nine or ten days. Gran¹⁹ found the *Bacillus gelaticus* produced sugar from agar-agar, which was rich in galactan. Saiki²⁰ obtained a slight gas production with *Bacillus coli communis* in media containing agar-agar and Irish moss (*Chondrus crispus*), both of which contain galactan. McCollum and Brannon,²¹ working with the intestinal bacteria of the cow, found they destroyed 28.09 to 76.13 per cent of the pentosan of corn fodder, wheat straw, and oat straw when anaerobic digestion was continued for fourteen days. Miss Swartz,²² while in Mendel's laboratory, experimented with numerous pentosans and galactans, finding the greater number resistant to bacterial action. Exceptions were the soluble dulse pentosan from the seaweed *Rhydomenia palmata* and the galactan of *Limu manaua*, a preparation from the Hawaiian seaweed *Gracilaria coronopifolia*. The dulse was readily decomposed by mixtures of either soil or faecal bacteria. The *Limu*

¹⁶ WINOGRADSKY: *Comptes rendus*, 1895, cxxi, p. 742.

¹⁷ BEHRENS: *Centralblatt für Bakteriologie*, 1902, ii, vii, p. 114.

¹⁸ SLOWTZOFF: *Zeitschrift für physiologische Chemie*, 1901, xxxiv, p. 181.

¹⁹ SWARTZ, MARY D.: *Transactions of the Connecticut Academy of Arts and Sciences*, 1911, xvi, p. 284.

²⁰ SAIKI: *Journal of biological chemistry*, 1906, ii, p. 258.

²¹ MCCOLLUM and BRANNON: *Journal of the American Chemical Society*, 1909, xxxiii, p. 1253.

²² SWARTZ: *Transactions of the Connecticut Academy of Arts and Sciences*, 1911, xvi, pp. 323-331.

manauca was resistant in all tests but one, and this was inoculated by exposure to air. This author found Irish moss entirely unaffected by common micro-organisms. Hence we may conclude that the agar-agar was fermented by *B. coli communis* in Saiki's experiment.

Bacterial digestion of the marc.—Preparations of the apple marc, cooked and raw; the residue of marc reclaimed after extracting marc several days with 0.4 per cent hydrochloric acid; and purified pectin were subjected to the action of intestinal bacteria. Two or 3 gm. of the marc or marc residue were usually suspended in 100 and 200 c.c. of water to which 1 per cent of Witte's pepton and a little sodium chloride had been added. The pectin media always contained 1 or 2 per cent of pectin, as well as the pepton. To these special media the bacterial cultures were added. Mixtures of intestinal bacteria were prepared by stirring fresh human fæces in sterile water and then allowing the larger particles to sediment. One half or 1 c.c. of this emulsion was then transferred to the special media. In one series of observations a pure culture of *B. coli communis* was used.

These mixtures were subjected to both aerobic and anaerobic methods of cultivation. The anaerobic culture observations were made with Novy's jars in which the air was replaced with hydrogen. All cultures were grown at body temperature. Control tests were usually made.

Early observations showed that reducing sugars very rarely appeared, and in the two instances where they were detected only traces were present. All cultures showed visible growth with some gas formation.

To determine what proportion of the sugar-yielding constituents was destroyed by bacteria, it was found most convenient to add enough hydrochloric acid to the mixture to make a 2 per cent solution, and then to hydrolyze it for four hours under a return condenser. After neutralization and filtration, the amount of reducing sugar was determined by the Allihn method and calculated as dextrose.

Table I summarizes the bacterial studies. The mixtures of faecal bacteria, both aerobic and anaerobic, destroy the hemicelluloses of the marc to a considerable extent. They attack the cooked marc more readily than raw marc. They also destroy the acid extraction residue with greater facility than they do raw marc. The seven-days experiment with the pectin makes it certain that faecal bacteria are capable of decomposing both the pentosan and galactan of apple

pectin, 66.4 per cent of the sugar-yielding radicles being destroyed. In no instance was the bacterial action allowed to continue long enough to determine whether the pentosan and galactan could be wholly destroyed.

TABLE I.
DESTRUCTION BY BACTERIA.

Preparation used.	Bacterial culture.	Days of growth.	Reducing sugars ¹ after hydrolysis.	
			Before cultivation.	After cultivation.
			per cent.	per cent.
Raw marc	Faecal aerobes	6	28.0	21.8
Raw marc	Faecal anaerobes	7	28.0	20.0
Cooked marc	Faecal aerobes	9	28.0	15.1
Cooked marc	Faecal aerobes	8	28.0	16.8
Acid extraction residue	Faecal aerobes	7	21.8	12.7
Acid extraction residue	Faecal anaerobes	7	21.8	13.9
Pectin	Faecal aerobes	3	57.45	37.7
Pectin	Faecal anaerobes	7	57.45	19.3
Cooked marc	<i>B. coli communis</i>	7	28.0	25.7
Acid extraction residue	<i>B. coli communis</i>	7	21.8	23.0

¹ Calculated as dextrose.

A visible growth of the *B. coli communis* was obtained in the two studies recorded. The results indicate that this organism was not the chief nor even an important agent of destruction in the cultures containing a mixture of faecal bacteria.

ENZYME STUDIES.

Bourquellot and Hérissé²³ found in malt diastase an enzyme capable of producing reducing sugar from pectin. They also report

²³ BOURQUELLOT and HÉRISSEY: *Comptes rendus*, 1898, cxxvii, p. 191, and 1899, cxxviii, p. 1241.

that a soluble ferment of *Aspergillus niger* partially hydrolyzed the pectose of gentian root, converting it into pectin.

Enzymes capable of hydrolyzing pentosans have not been reported to occur in higher animals. Slowtsoff,²⁴ working with xylan, observed it could be gradually hydrolyzed by 0.2 per cent hydrochloric acid, but was not digested by saliva, gastric, or pancreatic juice. Bergmann,²⁵ also working with xylan, found it to be unaffected by intestinal extracts of the hen, goose, guinea-pig, sheep, ox, and horse. Miss Swartz²⁶ found "Taka" diastase hydrolyzed the pentosan of dulse, but that dulse and the pentosan of limu lipoa, from the marine alga *Haliseria pardalis*, did not respond to saliva, malt diastase, pancreatic extract, intestinal extract, gastric extract, or 0.2 per cent hydrochloric acid.

Bierry, Giaja, Sawanura, Saiki, and Miss Swartz²⁷ have all failed to find galactanases in higher animals. Miss Swartz reports that "Taka" diastase hydrolyzed the galactan of the seaweed preparation limu kohu (*Asparagopsis sanfordiana*); and that 0.2 per cent hydrochloric acid hydrolyzed those of the marine algae preparations limu akiaki (*Ahnfeldtia concinna*), and limu kohu, and that of slippery elm.

Beaker trials with the apple marc and pectin.—Repeated digestions *in vitro* were tried with cooked and raw marc; with the residue obtained from marc extracted with 0.4 per cent hydrochloric acid; and the pectin. The enzyme preparations were (a) filtered human saliva, (b) diastase purified from Parke, Davis & Co.'s diastase by repeated precipitations with alcohol, (c) "Taka" diastase, also a Parke, Davis & Co. preparation that was freed from sugar by precipitation with alcohol, (d) extractions of dog's and pig's pancreas with 25 per cent alcohol, and (e) an alcoholic extract of dog's intestine. That all enzyme preparations were active was shown by control digestions. The digestive mixtures were kept at body temperature, protected from bacterial decomposition by toluene, from three to thirty days. Not a trial with marc, marc residue, or pectin showed a trace of reducing sugars when tested with Fehling's solution.

²⁴ SLOWTZOFF: *Zeitschrift für physiologische Chemie*, 1901, xxxiv, p. 181.

²⁵ SWARTZ, MARY D.: *Transactions of the Connecticut Academy of Arts and Sciences*, 1911, xvi, p. 277.

²⁶ SWARTZ: *Loc. cit.*, p. 285.

²⁷ SWARTZ: *Loc. cit.*, pp. 332-333.

The marc was slowly but incompletely hydrolyzed by a 0.4 per cent solution of hydrochloric acid at 38° C. Both pectin and reducing sugars were products of this hydrolysis. The proportions of each and the rate of formation are given in Table II. For the quantitative determinations the pectin was separated from the acid filtrate with alcohol as a clear gelatinous precipitate.

TABLE II.

	Day.	Pectin.	Sugar (as dextrose).
		per cent.	per cent.
3 gm. marc + 100 c.c. 0.4 per cent HCl.	1	6.03	2.67
3 gm. marc + 100 c.c. 0.4 per cent HCl.	2	8.73	1.85
3 gm. marc + 100 c.c. 0.4 per cent HCl.	4	10.26	4.30
3 gm. marc + 100 c.c. 0.4 per cent HCl.	5	10.73	4.22

Several attempts were made to separate all the pectin from the marc by the use of larger volumes of acid, but were not successful. The following is an example of such trials. Three gm. of the marc were hydrolyzed in a flask with 300 c.c. of 0.4 per cent hydrochloric acid, at body temperature, for four days, and the mixture was frequently shaken. After this the marc residue was hydrolyzed with 2 per cent hydrochloric acid under a return condenser and still yielded 14.16 per cent of reducing sugar which was calculated as dextrose.

DIGESTION AND UTILIZATION.

Lindsey, in 1904-1905, fed apple²⁸ pomace, with hay, to sheep and cows, and found 65.63 to 80.06 per cent of the total dry matter and 35.82 to 84.95 per cent of the fibre content was utilized. The sugar and hemicellulose content of the pomace were not determined. In the fibre, however, according to the analyses of marc by Bigelow, Gore, and Howard, there may be 40 per cent or more of pectin. With some of Lindsey's animals, therefore, a part of the pectin must have been

²⁸ LINDSEY: Seventeenth Annual Report, Massachusetts State Experiment Station, 1905, pp. 70, 86.

utilized. Alwood, Davidson, and Moncure²⁹ report apple pomace to contain as much, on the average, as 9.34 per cent of sugar. This in part accounts for Lindsey's observation that, "from the composition and digestibility of the pomace, it seems probable that 4 pounds, when fed in what is termed a 'balanced ration,' would be equal in feeding value to 1 pound of good cow hay."

A considerable number of pentosan feeding experiments³⁰ have been conducted with sheep, cattle, horses, rabbits, pigs, dogs, and fowl. All give evidence of a marked digestion of pentose-yielding hemicelluloses. We have Kellner³¹ to thank for the evidence that the digestion products are assimilated. He placed oxen in a respiration calorimeter and fed them starch and rye straw, rich in pentosans, and concluded that the pentosan of the straw took part in the formation of fat and indirectly in the formation of flesh.

Several researches have been conducted on man. König and Reinhardt³² found the pentosans of peas, red cabbage, beans, and Graham bread were largely utilized. They made no observations as to the influence of bacteria. Miss Swartz³³ administered marine algæ preparations, dulse, limu elele, and limu pahapaha, to human subjects, and observed that the pentosan of the dulse disappeared entirely from the intestine; that 69 per cent of the limu elele and 34 per cent of the limu pahapaha were utilized. It is to be noted that Miss Swartz earlier found that the pentosan of the dulse was readily decomposed by mixtures of faecal and soil bacteria. Therefore she attributed the disappearance of these pentosans in digestion to bacterial activity.

Researches dealing with the utilization of the galactans have been less numerous. Lindsey,³⁴ Lohrlich,³⁵ and Miss Swartz³⁶ have each shown them to be utilized to some extent by domestic animals.

²⁹ ALWOOD, DAVIDSON, and MONCURE: United States Department of Agriculture, Bulletin of the Bureau of Chemistry, 1904, No. 88, p. 13.

³⁰ See LINDSEY: Fifteenth Annual Report, Massachusetts State Experiment Station, 1903, pp. 75-81; also see SWARTZ: *Loc. cit.*, pp. 276-282.

³¹ KELLNER: Die Landwirtschaftliche Versuchs-Stationen, 1900, liii, p. 457.

³² KÖNIG and REINHARDT: Zeitschrift für Untersuchung der Nahrungs und Gemessmittel, 1902, v, p. 110, 1904, vii, p. 729.

³³ SWARTZ, MARY D.: *Loc. cit.*, pp. 344-348.

³⁴ LINDSEY: Seventeenth Annual Report, Massachusetts State Experiment Station, 1905, pp. 78-84.

³⁵ LOHRISCH: Zeitschrift für experimentelle Pathologie und Pharmakologie, 1908, v, p. 478.

Saiki,³⁷ while a pupil of Professor Mendel, was the first to try feeding experiments with galactans on man. He employed marine algæ preparations. Two diet periods with agar-agar gave coefficients of digestibility for the galactans of 8 and 27 per cent. With the Japanese wakame, from *Undaria pinnatifida*, 28 per cent was utilized; and with kombu, from *Laminariaceæ*, as much as 78 per cent was used. Lohrisch³⁸ studied the utilization of the galactan of "soluble agar" and found about 50 per cent was digested. He further conducted three respiration experiments on men and obtained a rise in respiratory quotient which indicated that this carbohydrate is oxidized in the human body. Miss Swartz³⁹ found on feeding Irish moss to human subjects that one subject was unable to use any of the galactan, while another utilized 11 per cent. The coefficient of digestibility for the galactan of *limu huna* was 10 per cent, and for *limu akiaki* 60 per cent. This poor utilization of these galactans was in accord with the high degree of resistance these seaweed preparations showed to bacterial decomposition.

Digestibility of the apple marc.—Human subjects served in these experiments, and throughout the entire period of observation used a diet practically free from cellulose. White bread, crackers, butter, cream, milk, cheese, meats, eggs, sugar, and coffee were taken. While the food was not weighed, yet approximately the same quantity of each article of diet was used each day. When marc was added to the daily diet, no reduction was made in the amounts of other foods. It was served as a cereal with sugar and cream. For the cooked marc period it was cooked in a water bath for an hour and a quarter.

The feces of each period were marked off with lamp-black, taken in gelatin capsules just before the first meal of the period. Each collection of feces was immediately weighed and at once mixed with alcohol and then dried over a water bath. The amount of hemicelluloses remaining unutilized was determined by boiling 3 gm. of the air-dried, thoroughly mixed, powdered feces with 100 c.c. of a 2 per cent hydrochloric acid for four hours and then the sugar test applied as in other hydrolysis experiments.

³⁶ SWARTZ, MARY D.: Transactions of the Connecticut Academy of Arts and Sciences, xvi, p. 352.

³⁷ SAIKI: Journal of biological chemistry, 1906, ii, pp. 261-262.

³⁸ LOHRISCH: *Loc. cit.*

³⁹ SWARTZ: *Loc. cit.*, p. 353.

TABLE III.
DIGESTIBILITY OF APPLE MARC.

No.	Subject.	Period.	Diet.	Composition of faeces.				Hemicellulose fed (as dextrose), gm.	Coefficient of digestibility.
				Weight moist, gm.	Weight air dry, gm.	Hemicellulose (as dextrose), per cent.	Hemicellulose (as dextrose), gm.		
1	Man	Fore = 2 days	Cellulose free	137.5	30.4	3.33	1.01
		Mid = 2 days	Same + 40 gm raw marc	214.0	40.11	6.88	2.76	11.2	84.5
		After = 2 days	Same as fore	133.2	31.44	3.33	1.04
2	Woman	Fore = 2 days	Cellulose free	65.5	22.5	3.52	0.79
		Mid = 2 days	Same + 40 gm raw marc	280.5	61.0	4.19	2.56	11.2	83.1
		After = 1 day	Same as fore	45.0	7.0	4.92	0.34
3	Man	Fore = 2 days	Cellulose free	161.5	34.5	3.19	1.10
		Mid = 2 days	Same + 60 gm raw marc	331.5	63.5	6.98	4.44	16.8	79.1
		After = 2 days	Same as fore	107.5	27.0	3.26	0.88
		Mid = 2 days	Same + 60 gm cooked marc	402.5	64.5	6.22	4.01	16.8	81.3
		After = 2 days	Cellulose free	99.0	27.5	3.03	0.84

The data obtained from these feeding experiments are tabulated in Table III. The coefficients of digestibility are higher than the bacterial observations on the raw marc alone would lead one to expect, but accord better with the destructions of the hemicelluloses obtained through the action of bacteria on the acid treated, the cooked marc, and the pectin. It is clear, then, that the action of the gastric juice

TABLE IV.

Carbohydrate.	In faeces.		Adminis- tered.	Coefficient of digestibility.
	per cent.	gm.	gm.	
After hydrolysis, as dextrose . . .	6.22	4.01	16.8	81.3
Pentosan	2.95	1.90	16.81	88.69
Galactan	2.50	1.61	6.94	76.78

on the marc favors its bacterial decomposition in the intestine. Furthermore, cooking the marc does not materially increase the amount of destruction it undergoes during digestion.

The faeces of the cooked marc period of subject No. 3 were also analyzed quantitatively for the galactan and pentosan. The results are given in Table IV. Both of these carbohydrates very largely disappear during the journey through the digestive canal. The preliminary study of the action of bacteria on the pectin had suggested the possibility that both the pentose and galactan radicles of the pectin would be destroyed by bacteria in the process of digestion. Undoubtedly their disappearance in digestion, because of the absence of pectin-digesting enzymes, must be attributed to the activity of intestinal bacteria.

The urine during a marc period failed to give tests for reducing sugars and also gave a negative test for pentose. No examinations were made for either pentosan or galactan.

It will be observed in Table III that in the marc periods the apple marc increased materially the total amount of faeces. The faeces, however, were so well formed that it hardly seems probable that the marc contains those constituents of the apple which cause looseness of the bowels in many persons after eating apples.

SUMMARY.

1. The pectin prepared from apple marc by hydrolysis with hydrochloric acid yielded 35.87 per cent of pentosan and 45.83 per cent of galactan.

2. Mixtures of intestinal bacteria destroy the hemicelluloses of marc and also destroy pectin. They do not form reducing sugars as an end product. They destroy almost equally well the pentosan and galactan of the pectin.

3. Animal enzymes, malt diastase, and "Taka" diastase do not hydrolyze the marc or its pectin.

4. Weak solutions of hydrochloric acid split off from the marc pectin and reducing sugars.

5. From 79.1 to 84.5 per cent of the hemicelluloses of the marc is either utilized or destroyed during digestion. Bacterial activity probably accounts for this disappearance. The coefficient of digestibility for the pentosan was 88.69 and for the galactan 76.78.

The writer wishes to express his hearty thanks to Professor Lafayette B. Mendel for suggestions and advice.

ELECTRICAL CHANGES IN THE HEART DURING VAGUS STIMULATION.

By WALTER J. MEEK AND J. A. E. EYSTER.

[From the Physiological Laboratory of the University of Wisconsin.]

ALTHOUGH attempts had been made previously, Gaskell¹ was the first to report electrical changes in the heart during vagus action. His experiments were made possible by the peculiar anatomy of the terrapin's heart which has a branch of the vagus running free with the coronary vein from the sinus to the posterior base of the ventricle. The sinus and left auricle could therefore be cut away from the rest of the heart while the vagus connections were maintained with the ventricle and the right auricle. This was practically a first Stannius' ligature, and it was usually followed by a period of quiescence in the right auricle and the ventricle. During this period Gaskell found that the demarcation current from the burned tip of the right auricle and the uninjured base of the same chamber showed a positive variation on vagus stimulation. The support that this experiment gave to Gaskell's assimilatory theory of inhibition and the part this has played in physiology are matters too well known to merit discussion here.

So far as we can find, the literature does not contain a single paper describing an exact repetition of Gaskell's experiments. Boruttau,² however, in an oral report to the Deutsche physiologische Gesellschaft in 1905, stated that only in the case of the terrapin's auricle had he been able to get a positive variation of the demarcation current such as that described by Gaskell. Boruttau had made attempts with the auricles and ventricles of frogs and terrapins. No other worker to our knowledge has reported definitely for or against the original experiments.

¹ GASKELL: Beiträge zur Physiologie, Festschrift zu Ludwig, 1887, p. 114.

² BORUTTAU: Zentralblatt für Physiologie, 1905, xix, p. 301.

From time to time, however, workers using other galvanometers have failed to find electrical changes during vagal stimulation such as those originally described. Gotch³ used the capillary electrometer on a tortoise's auricle prepared in Gaskell's way. The electrometer showed monophasic variations of the demarcation current corresponding to each beat, but on vagus stimulation the meniscus came to rest at exactly the same point it had occupied during the previous diastoles. Burdon-Sanderson,⁴ in commenting on this demonstration, insisted that there still might have been positive variations which even the sensitive electrometer failed to detect. Fano and Fayod⁵ reported rhythmical variations of the capillary electrometer which were more or less independent of the regular auricular beats. These variations changed in character during vagus stimulation, and the authors thought there was some evidence in this of a trophic function of the vagus as advocated by Gaskell.

Einthoven,⁶ in 1908 while working with the string galvanometer, was also impressed with the fact that during vagus stimulation no electrical changes appeared in the mammalian electrocardiogram which could be interpreted as positive variations. In numerous records he found that the position of the string indicated no change in potential during slowing of the heart, yet this was to be expected if the basal portions became more positive. Einthoven reviewed Gaskell's work, pointed out the inefficiency of the instruments employed, expressed doubt concerning the original investigations, and concluded that in the dog at least there was no evidence of an increase in positivity during vagus stimulation. Kahn⁷ made the same observations on electrocardiograms as Einthoven and drew much the same conclusions.

Our interest in the problem grew from studying electrocardiograms taken during vagus inhibition in various experiments we had made on cats and dogs. These records all confirmed the observations of Einthoven and Kahn that there was no change in the mean level of

³ GOTCH: *Journal of physiology*, Proceedings of the physiological society, 1887, viii, p. xxvi.

⁴ BURDON-SANDERSON: *Ibid.*, p. xxvii.

⁵ FANO and FAYOD: *Archives italiennes de biologie*, 1888, ix, p. 143.

⁶ EINTHOVEN: *Archiv für die gesammte Physiologie*, 1908, cxxii, p. 517.

⁷ KAHN: *Archiv für die gesammte Physiologie*, 1909, cxxvi, p. 197.

the string during vagus action. We then determined to repeat Gaskell's experiment on the tortoise, using in place of the D'Arsonval the large Einthoven string galvanometer. The string galvanometer which detects extremely minute currents and responds accurately to the most rapid changes in potential would seem to be the ideal instrument for such an investigation.

The tortoises were prepared in the manner described by Gaskell. The species used was *Chrysemis concinna*. The coronary nerve was easily found, and a ligature laid in such a way that on being tied it spared the nerve but severed the right auricle and ventricle from the sinus and left auricle. This was found more satisfactory than severing the parts with scissors, although the latter method was used in the first experiments. The tip of the right auricle was seared with a hot glass rod. Non-polarizable electrodes were placed on the burned apex and uninjured base of the auricle. The vagus was isolated and tested with medium-strength tetanic stimuli from a Du Bois Raymond coil. The sinus and left auricle which had continued to beat were of course inhibited if the vagus remained active.

In our first experiments we had difficulty in securing quiescent preparations. Either the stimulus caused by cutting off the sinus and left auricle or by stretching the right auricle to accommodate the electrodes seemed to develop automaticity, and the period of quiescence was much shortened or entirely absent. We found, however, that terrapins differ greatly in this regard. In a later shipment we secured a typical quiescent preparation from every specimen, automaticity developing, if at all, only after several hours. Even in those cases in which automaticity had developed it seemed that any change of potential due to vagus activity should manifest itself, since the string of the galvanometer responds almost instantly and the general change in level would not therefore be obscured by the beats superposed on it. The demarcation current which appeared on connecting up the electrodes was compensated and the shadow of the string brought upon the slit of the photographing apparatus. The electrodes were so placed that an up stroke indicated positivity of the auricular base. Records were then taken during which the vagus was stimulated from two to fifteen seconds. The results were uniformly negative. In those cases in which the preparation was automatic, the contractions of auricle and ventricle were marked by beats, but the mean

level of the string never changed throughout the record. In those cases in which the preparation was quiescent the beats of the left auricle disappeared during stimulation, but the mean level of the string, as before, remained unchanged.

In the experiments just mentioned the sensitiveness of the galvanometer was such that 1 millivolt deflected the shadow of the string on the slit through a space of 27 mm. Although this might be consid-

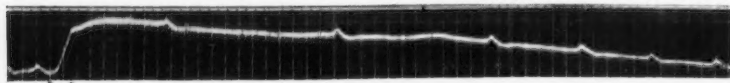


FIGURE 1. — About one fourth the original size. Increase in positivity at base of right auricle during stimulation of right vagus. Tetanic stimulation of vagus from X to X. Time at top of record in seconds. Beats recorded by string due to regular contractions of left auricle. Electrodes on seared apex and uninjured base of right auricle, and so placed that positivity of auricular base gives upward movement of string. Sensitiveness of string such that 1 millivolt gives a deflection of 270 mm.

ered a fairly sensitive string, it occurred to us that possibly we were really overrating its sensitiveness. Such proved to be the case. We found that 1 millivolt gave a deflection in the laboratory D'Arsonval of 620 mm. Assuming that Gaskell worked with an instrument of this sensitiveness in the cases in which he secured variations of 20 mm., we could expect a variation of only 1 to 2 mm. in the string galvanometer. This indicated that our string was by no means sensitive enough for the object in view. A quartz thread was then inserted and the tension loosened until 1 millivolt gave a deflection of 1350 mm. Our results at once became uniformly positive. Experience showed that we could secure positive variations on vagal stimulation with a deflection of the string anywhere from 270 to 1350 mm. With the latter sensitiveness the reaction was usually so marked that the shadow of the string passed entirely out of the range of the photographing apparatus.

In Fig. 1 we have the results of stimulating the right vagus in a quiescent Gaskell's preparation. The time was marked in one-second intervals at the top, and the duration of stimulation by indentations at the bottom of the record. The position of the secondary coil was 5.3 cm. The electrodes were so placed that an upward movement indicated positivity of the auricular base. The sensitiveness of the string was such that 1 millivolt gave a deflection of 270 mm. It will

be seen that within one second after the vagus was stimulated the string began to rise rapidly. At the end of five seconds it had reached a height of 35 mm. Within three seconds it began to return slowly to its former position. The return was much slower than the initial rise, and the original level was not reached until the end of the record, about sixty-three seconds. The beats appearing on the record were due to the left auricle, which being in connection with the sinus had maintained its rhythm. The chronotropic action of the vagus can be seen in the slowing of this rhythm.

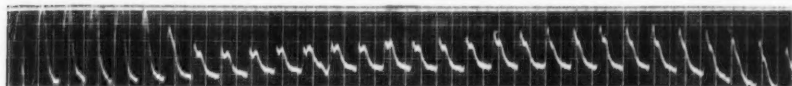


FIGURE 2. — One fourth the original size. Increase in positivity at base of right auricle during stimulation of left vagus. Sensitiveness of string such that 1 millivolt gives a deflection of 1150 mm.

In six terrapins examined with the very sensitive string we never once failed to demonstrate an increased potential at the base of the auricle on stimulation of the right vagus. The effect appeared on the average at the end of one second after stimulation. The return to the original condition occurred in from twenty-three to seventy seconds. The exact increase in potential could not be measured in all cases because the shadow of the string usually passed out of range of the photographing apparatus. It was evident that it varied greatly with the different animals. In Fig. 1 the increase in potential can be accurately estimated. It is something over 10^{-4} volt.

In four of the six terrapins we found the left vagus gave results similar to the right. Fig. 2 is a record of such an experiment. The stimulus lasted twenty seconds. There was only a slight chronotropic effect, but rather a marked inotropic. The diastolic level of the thread rose about 10 mm., indicating increased positivity at the base of the right auricle. In no case did we find the left vagus as active as the right, and in two of the six experiments we were unable to secure any results from the left vagus even with the most sensitive string which we were able to use. Recent work by Garrey⁸ has shown that the vagi of the turtle have a preponderant homolateral effect,

⁸ GARREY: this Journal, 1911, xxviii, p. 330.

but that crossed results may be obtained with strong stimuli. Our experiments agree with Garrey's findings.

In comparing our work with that of Gaskell it is evident that the string galvanometer has almost exactly duplicated the results he obtained with the D'Arsonval. Our method has had the advantage of being a graphic one. Gaskell found that the increase in potential took place almost immediately after stimulation and in every case had reached its maximum within ten seconds. Only in one or two of his experiments did the increase in potential last as long as one

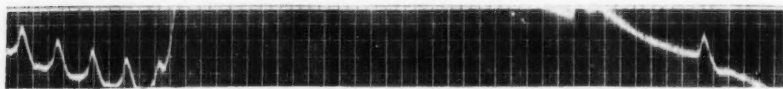


FIGURE 3. — One fourth the original size. Record showing gradual decrease in the demarcation current and also the effect of stimulating the right vagus tetanically. Sensitiveness of string such that 1 millivolt gives a deflection of 500 mm.

minute. In all these details our own results are in close agreement. The rate of decrease in the potential at the auricular base after its rapid rise due to the stimulation is much slower in our records than that observed by Gaskell. As noted by Gaskell, the demarcation current gradually decreased as the experiments progressed. Fig. 3 shows this decrease as well as the effect of stimulating the right vagus. The thread was very sensitive, and part of the shadow escaped the recording apparatus.

Gaskell was convinced that he was dealing with a physiological property of the vagus because he failed to secure similar effects on stimulating the accelerators. Spread of current was ruled out of our experiments in several ways. The left vagus did not always give results. In time the effect of the right vagus wore off and finally disappeared. Stimulating the carotid artery in exactly the same way that the vagus had been stimulated was ineffective. It would seem beyond question that the increased positivity exhibited by the auricular base during vagus stimulation is a true physiological action brought about by that nerve.

Our experiments make it apparent why Einthoven and Kahn have failed to find increased positivity in the mammalian heart on vagus stimulation. In mammalian work it is impossible to use a string

sensitive enough to record the increased potential. A string sufficiently sensitive to have its shadow deflected 270 mm. by 1 millivolt at the distance of 1 meter would of course be broken by the action currents of the mammalian heart. This difficulty can hardly be overcome. Previous workers have evidently overestimated the sensitiveness of their string as compared with the type of galvanometer used by Gaskell, and also the amount of potential increase actually brought about by vagus stimulation.

SUMMARY.

1. Gaskell's observations showing an increased potential in the auricular base of the terrapin's heart during vagus stimulation have been repeated with the string galvanometer. The increased positivity of the auricle appears within one second after stimulation begins, reaches its maximum in three to five seconds, and then gradually returns to the original value during the next twenty-three to seventy seconds.

2. The right vagus seems to be active in all preparations. The left vagus influenced the right auricle in four out of six terrapins, but the effect was less marked than that obtained by the same stimulation of the right nerve. The innervation of the vagus therefore seems to be mostly homolateral.

3. The extreme sensitiveness of the string required to demonstrate this so-called trophic action of the vagus makes it improbable that the method can be applied to the mammalian heart.

ON THE ABSORPTION OF FAT BY THE SALMON STOMACH.¹

BY CHARLES W. GREENE.

[From the Department of Physiology and Pharmacology, Laboratory of Physiology,
University of Missouri.]

MARCET² in 1858 published experiments on dogs showing that after a meal consisting of meat and mutton fat the content of the stomach yielded in every case large amounts of fatty acids though the fats of the food were neutral. This was the first demonstration that fats were dissociated in the stomach. Cash³ in 1880 observed the digestion of fat in the stomach. He was also able to secure an increased amount of fatty acids when he subjected neutral fats to the action of extracts of the gastric mucous membrane in the presence of hydrochloric acid. After removing the pancreas to eliminate the pancreatic juice known to contain lipase, there was an abundant absorption of fats, as shown by the abdominal lacteals. From these various tests Cash drew the conclusion that the gastric secretion contained an active lipase. The work of Cash was supported by Ogata,⁴ who devised an experiment to test the fat-digesting action in the stomach itself. Cash made the significant observation that the gastric mucous membrane contained both neutral fat and fatty acids, though he attached no importance to this observation. Harley⁵ in 1897 showed that neutral fats were dissociated in the stomach in dogs in which the reflux of pancreatic juice was made impossible by removal of the gland. The percentage of fatty acid in the stomach was found to be as high as 31.29 per cent of the total

¹ Published by permission of the U. S. Commissioner of Fisheries.

² MARCET: *Medical times*, 1858, xvii, p. 209.

³ CASH: *Archiv für Physiologie*, 1880, pp. 323-333.

⁴ OGATA: *Ibid.*, 1881, p. 515.

⁵ HARLEY: *British medical journal*, 1897, i, p. 1218.

⁶ VOLHARD: *Zeitschrift für klinische Medicin*, 1900, xlii, p. 414, and 1901, xliii, p. 613.

fats introduced. Volhard⁶ took up the question of the action of gastric juice on neutral fat. In his classic series of papers he gives much valuable detailed experimental evidence which tends to place beyond further doubt the fact of the digestion of fats in the gastric cavity and under the influence of the gastric juice.

The experimenters who have striven to test the digestion of fats in the stomach have given no attention to the possible absorption of fat cleavage products in this organ. The status of our knowledge of this question was expressed by Noël Paton,⁷ in 1911, in the following words: "There is no evidence that fats are absorbed under any condition in the stomach." Nevertheless the literature shows that in 1901 Schilling⁸ observed by histological methods that fat was absorbed into the epithelium of the stomach of the sucking calf. He found fat in smaller quantity in those animals killed some time after feeding. In a calf killed after a rich meal of milk the epithelial cells in the middle stomach region were thickly filled with microscopic fat drops. The fat had penetrated into different levels of the epithelium. This has been accepted as a demonstration of the taking up of fat by the gastric mucosa.

I have demonstrated the absorption of fat by the stomach of the young king salmon, *Oncorhynchus tshawytscha*. While pursuing my general physiological studies of this species in California the opportunity came to me to test how these fishes take up the fats of their food. The intestine and numerous pyloric cæca were found to absorb fats with avidity and in large quantity. But it was a surprise to find an active fat absorption taking place through the cylindrical epithelium forming the superficial coat of the gastric mucosa.

The method used was to feed olive oil to the salmon, keeping the fish in an aquarium. The alimentary tube of the salmon is quite simple in its gross anatomy. It is an S-shaped tube, with no complex features other than the large number of blind sacs, or cæca, at the pyloric end of the intestine. In practice it was found to be an easy matter to inject oil from a medicine dropper into the rectum. A gentle pressure continued for a few moments will press the oil entirely through the canal until it flows from the mouth. Giving the oil by way of the mouth is not so satisfactory.

⁷ PATON, D. NÖEL: *Encyclopedia Britannica*, 1911, xix, p. 925.

⁸ SCHILLING: *Fortschritte der Medicin*, 1901, xix, p. 613.

The absorption was tested by Bell's modification of the Herxheimer method of staining the fats in frozen microscopic sections. It was assumed that any fat absorption by the stomach



FIGURE 1.—Gastric superficial epithelium of fat-fed salmon No.46 from Brookdale Hatchery, California. This young salmon was fed olive oil 42 hours before killing. The material was fixed in Flemming's stronger solution. The fat has penetrated the outer zone of the superficial cells and the cells of the crypts as deep as the neck cells. The Osmic staining shows a relatively greater number of fat droplets in the bodies of the cells in portions of the section not shown in the figure. This figure is typical of the early stage of fat absorption. Camera outlines. Magnification, Leitz ocular 1, objective 7.

mucosa would be by the same mechanism operative in the intestine. Therefore one might expect a resynthesis of fats in the absorbing epithelium. It was found that fats were deposited in the gastric epithelium, though the nature of the fats was not determined further than that they stain both with osmic acid and with scarlet red.

Fat absorption in different regions of the stomach.—The usual cardiac and pyloric divisions of the stomach are sharply defined in the salmon. The cardiac division is abundantly supplied with peptic glands of the tubular type usual with fishes. The surface of the mucosa is covered with rather long but slender cylindrical epithelial cells.

The pyloric end of the stomach is smaller and ends abruptly in a valve-like constriction that separates it from the pyloric portion of the intestine. The lining mucous coat is thrown into deep folds, but no glands are present. The cylindrical epithelial covering is only slightly differentiated. The outer or more superficial cells are relatively long and cylindrical, though not so long and slender as in the cardiac end. The cells of the deep folds are more nearly cubical. There is no evident differentiation of structure, and the deeper cells bear no resemblance to gland cells.

The absorption of fat by the cardiac division of the stomach.—The superficial epithelial cells of the cardiac stomach contain an unusually large number of fat droplets after fat feeding.

These cells are free of fat in the fasting salmon, and the fat drop-

lets are few in number, and extremely small in size in an animal that is feeding naturally.

The fat droplets vary greatly in size. They vary from a fraction of a micron to two micra in diameter. They are most numerous in the most superficial epithelium, but are present as deep as the neck cells. Fish were killed at different times after feeding, from ten hours to forty hours and more. The fat appears in the earlier stage of absorption crowded into the outer or free ends of the cells (Fig. 1).

At a later stage the fat drops are distributed along the body of the cell and around the nucleus. Still later the fat is massed about the bases of the cells. The fat drops vary in size in different parts of the cell, small at the free border, larger at the central portion, and again small at the basal part of the cell.

The fat passes through the body of the cells and not through the cement substance between the cells. Little fat was found in the supporting connective tissue, a fact taken to indicate a relatively rapid removal of the fat after it passes through the cylindrical cells.

Absorption of fat from the pyloric division of the salmon stomach.—The epithelial covering of the pyloric part of the stomach is comparatively simple and undifferentiated. The membrane is more folded near the bend of the stomach and more and more simple toward the pylorus. The most superficial folds have somewhat longer cells.

The sections made at a favorable time after feeding fat show the superficial epithelial cells crowded with fat. Ofttimes the cells are filled from tip to base, so full of fat droplets that all structural detail is obscured. Not all folds are equally loaded with fat. Some will have the surface cells filled throughout, other folds will have the free ends filled and the bases of the cells sparsely dotted with fat droplets. In



FIGURE 2. — Two fifths the original size. General view of a section of the pyloric division of the stomach of fat-fed salmon No. 88, McCloud River, Baird, California. The section was cut near the pyloric valve. Most of the deeper folds are free of fat, but two have fat in the mucosa for their full depth. Only a trace of fat was found in the connective tissue supporting the mucosa.

such instances the droplets at the bases of the cells are smaller than at the apex, as indicated in the figure submitted.

Occasionally all the cells lining a fold will contain fat, even down to the bottom of the fold. Two such folds are shown in the figure. The amount of fat in the cells is less and less the deeper in the fold the examination is made. A large portion of the deeply folded mucosa contains no fat. This fact is not due to any lack of ability of these cells to absorb. Rather is it due to the fact that the fat is not brought in sufficiently intimate contact with the deep folds of the mucosa. The mucous surface extending deepest into the cavity of the stomach is brought in most intimate contact with the fats of the food. These cells are the ones most loaded with fat.

The fat droplets in the pyloric epithelium are larger than in the cardiac epithelium. The droplets in the neck cells and in the bottoms of the crypts of the pyloric region are smaller than in the more superficial cells. The size and number of fat droplets probably vary in relation to the rate at which absorption and resynthesis is taking place. This deduction is supported by the observations made on the pyloric cæca of the same salmon. The cæca are special fat absorbers. The fat is taken up in very much greater quantity than in the gastric epithelium. In the cæcal cells the fat droplets are often three times greater in diameter than in the gastric cells.

My observations show that the gastric absorption of fat does not nearly equal the intestinal absorption in amount and volume. But the observations do show conclusively that the stomach is a fat-absorbing organ of no insignificant proportions. The final report of these experiments will be published in the Bulletin of the U. S. Bureau of Fisheries.

COMPRESSION OF THE CARDIAC NERVES OF LIMULUS, AND SOME ANALOGIES WHICH APPLY TO THE MECH- ANISMS OF HEART BLOCK.¹

By WALTER E. GARREY.

[From the Physiological Laboratory of Washington University, St. Louis, and the Laboratory
of the U. S. Bureau of Fisheries,² Beaufort, N. C.]

INTRODUCTION.

THE experimental work which will be described in this paper was undertaken with the view of ascertaining the effects of compression upon conduction in nerves. The chief object in view was to determine whether the changes in conduction in the nerves of the heart of *Limulus polyphemus* were comparable to the changes in the atrio-ventricular bundle of the mammalian heart when clamped by the method of Erlanger.³ It was believed, and this belief has been justified, that such an investigation would throw further light on the mechanisms of heart block.

The effects of compression upon conduction in motor nerves have been noted in numerous investigations. In recent years Ducceschi⁴ has made the most extensive observations, while the work of Meek and Leaper⁵ merits especial attention owing to the refinement and accu-

¹ These investigations were reported to the American Physiological Society, December 29, 1911, at which time numerous tracings illustrating the details were reproduced. A report was also made to the St. Louis Medical Science Club, January 9, 1912. The paper is abstracted in this Journal, 1912, xxix, Proceedings of the American Physiological Society, p. xxi.

² I am indebted to the Commissioner, Hon. Geo. M. Bowers, for the opportunity of conducting these investigations, and to Mr. H. D. Aller, Director of the Beaufort Laboratory, for his co-operation in placing at my disposal the conveniences and facilities for prosecuting the work.

³ ERLANGER, J.: *Zentralblatt für Physiologie*, 1905, xix, p. 9, and *Journal of experimental medicine*, 1906, viii, pp. 8-58.

⁴ DUCCESCHI: *Archiv für die gesammte Physiologie*, 1901, lxxxiii, p. 38.

⁵ MEEK and LEAPER: this Journal, 1911, xxvii, p. 308.

rate quantitative character of the method employed by them, and owing to the further fact that they also hoped to throw light upon the nature of the conducting tissue in the atrio-ventricular bundle. While Meek and Leaper found only a slight difference between the amount of pressure required to block impulses in motor nerves and in skeletal muscle, it is none the less highly probable that quantitative differences in the reaction to compression between the types of conducting tissue extant in the vertebrate heart do exist. This view is based upon a previous communication by the author,⁶ in which it was shown that it requires a greater degree of compression to block inhibitory nerve impulses than normal autogenous impulses in the turtle's heart. One is led, from this and other facts, to expect that, qualitatively at least, all types of conducting tissue comport themselves in a similar manner when compressed. It is not the least surprising, therefore, that the results of our experiments upon nerves are, in the main, in harmony with the previous findings in experiments upon the auriculo-ventricular bundle of *His*, showing only such deviations as are to be expected from tissues with the physiological properties found in the *Limulus* heart.

THE METHOD.

a. Material. — The heart of *Limulus polyphemus* ("King crab," "Horseshoe crab") was chosen as most suitable for this investigation. As has been pointed out by Carlson,⁷ the impulses in this heart are neurogenic. They originate in a ganglionic nerve cord which is situated on the dorsum of the tubular, segmented heart. The chief mass of the ganglion lies upon the posterior segments. The impulses are transmitted to the anterior segments by nerves exclusively. There are three of these nerve paths: the anterior prolongation of the ganglionic cord and the two lateral or marginal nerves. This anatomical arrangement makes it possible to cut or clamp each or all of the conducting nerve paths. The myocardium is syncytial, and arranged circularly in nine segments. The only physiologic connection between these segments is to be found in the nerve paths just mentioned. The myocardium contracts, like skeletal muscle, with a force graded to the

⁶ GARREY, W. E.: this Journal, 1911, xxviii, p. 249. Also FREDERICQ, L.: Archives internationales de physiologie, 1912, xi, p. 405.

⁷ For literature concerning the heart of *Limulus*, cf. CARLSON, A. J.: Ergebnisse der Physiologie, 1909, viii, p. 373.

strength of the impulses which reach it. It is therefore possible to get graphic records which show the results of compression.

b. **Procedure.** — The conditions under which this investigation was prosecuted did not admit of the use of the liquid transmission method employed by Meek and Leaper. In its stead a modified Gaskell clamp, open at one side, the jaws of which were covered with thick smooth pure gum tubing, was employed. In the earlier experiments these elastic pads were not used, but were resorted to as soon as it became evident that it would otherwise be impossible to graduate the amount of pressure with sufficient accuracy, or to avoid permanent injury to the nerves. For adjustment and graduation of pressures the clamp was provided with a very fine micrometer screw. The clamp was placed in most instances at about the junction of the second and third segments. This location was chosen because the bulk of the rhythm-initiating nerve cord is posterior to this point, and because after section, or clamping to complete block, at this point, the anterior segments only rarely initiate an automatic rhythm. Various procedures were resorted to in the application of the clamp. In some instances the whole cross section, including the muscle tissue and the three nerve paths, was compressed. In other instances the lateral nerves were cut and the central nerve cord only was clamped; or again, the nerve cord was cut or dissected back from the anterior segments, and the clamp applied to both lateral nerves or to one of them after section of the other. The results are identical in all these procedures as far as the effects of compression are concerned, being modified only by the fact that the height of contractions of the anterior segments is much decreased by any procedure which materially decreases the number of nerve fibres conducting impulses to their musculature. Although many experiments were performed upon the heart *in situ*, it was found to be more convenient and satisfactory to work upon the excised organ immersed in its own plasma or in sea water. The latter method was therefore most generally used.

A light heart lever was attached to a muscle segment posterior to the clamp (usually the sixth); its movements served as an index of the strength of the impulses sent out by the nerve cord. The movements of a second lever attached to a segment anterior to the clamp afforded an accurate measure of the effects of compression. In such a preparation the posterior segments may be likened to the auricles and the an-

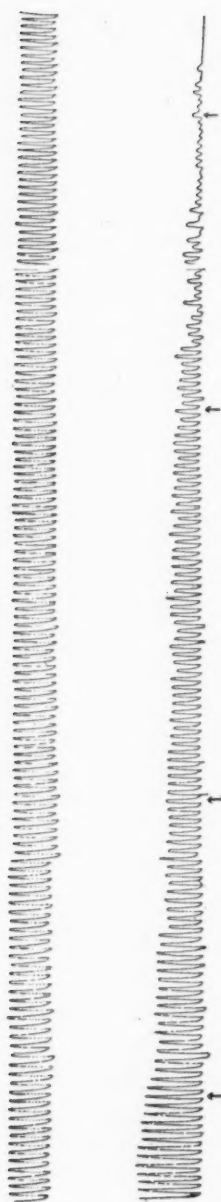


FIGURE 1. — Shows the weakening of impulses and their irregular strength resulting from compression of the nerve cord between the second and third segments. Upper tracing from the sixth segment. Lower tracing from the second segment anterior to the clamp, which was tightened at the points marked with arrows. Rate = 20 beats per minute. About two thirds the original size.

terior segments to the ventricles; the conducting nerve to the atrioventricular bundle.

GENERAL RESULTS OF COMPRESSION.

The most striking effect of the gradual application of compression to one or all of the conducting nerves was a reduction in the height of contraction of the muscle of the anterior segments, distal to the clamp. When the compression was very gradual, the reduction in the contractions was also very gradual and perfectly uniform (Fig. 1). When the compression was induced by more rapid approximation of the jaws of the clamp allowing an interval of time between successive manipulations, the tracings presented a series of step-like reductions in the height of contraction, each step corresponding to an increase in pressure (*cf.* Fig. 6). With the application of a given degree of compression, the major effect is immediate, but the appearance of the full effect requires the lapse of a certain time, during which, if the pressure is maintained constant, the height of the contraction gradually decreases. The importance of a similar time factor has been noted by Ducceschi and by Meek and Leaper (*loc. cit.*) in their observations upon compression of motor nerves.

When a given amount of pressure had been so graded that complete block was just obtained, and the pressure was then removed, the nerves gradually,

and in many instances completely, recovered their conductivity and the subsequent contractions reached their original height. While recovering, however, as well as during the application of the severer grades of pressure, the individual contractions may show considerable variations in height, a fact to which detailed reference will be made later. Occasional instances were noted in which after the contraction had reached a minimum a partial recovery ensued, although the compression was maintained constant. This recovery during compression never resulted if the degree of compression was severe. When the severer degrees of compression were sustained for some time, only partial or no recovery ensued, a permanent injury to the nerves having resulted.

Analysis of results.—The weakened contractions are the result of alterations in the conductivity of the nerves at the point of compression, which demand further analysis. Carlson⁸ has shown that stimuli applied to the lateral nerves or to the anterior portion of the nerve cord may cause an inhibition of the ganglionic impulses in the *Limulus* heart, and this finding has been confirmed by the author. Ducceschi⁹ and others have also shown that compression may stimulate motor nerves mechanically. Such mechanical stimulation with resultant inhibition could explain a weakening of ganglionic impulses, but does not account for our findings as recorded above, for examination of our tracings shows that the contractions of the anterior segments distal to the clamp are the only ones affected by the compression. There was no inhibition of the ganglionic impulses, since the contractions of the segments posterior to the clamp showed no alteration in rate, rhythm, or strength. Our results can be attributed solely to alterations in conductivity of the conducting nerve fibres at the site of clamping.

COMPARISON OF COMPRESSION WITH SECTION OF THE CONDUCTING NERVES.

From the very nature of the method employed some nerve fibres must inevitably have been more affected by the compression than

⁸ CARLSON, A. J.: *Ergebnisse der Physiologie*, viii, p. 434, and this Journal, 1905, xiii, p. 229.

⁹ ZEDERBAUM: *Archiv für Anatomie und Physiologie*, 1883, p. 161; EFRON: *Archiv für die gesammte Physiologie*, 1885, xxxvi, p. 467; DUCCESCHI: *Loc. cit.*; MEEK and LEAPER: *Loc. cit.*, Fig. 2, p. 317.

others. It seemed possible, then, that our results might be explained by assuming that the conductivity of some of the nerve fibres was completely suppressed while other nerve fibres were still able to conduct normally. To test this possibility Carlson's¹⁰ experiments on the section of the nerves were repeated with certain variations. It was found, in confirmation of Carlson's work, that a decrease in the number of conducting nerve fibres by section decreased the height of contraction

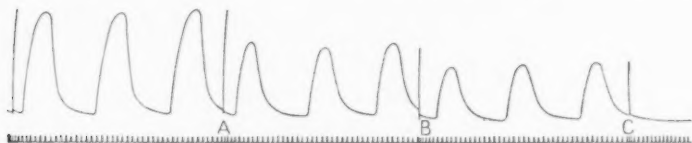


FIGURE 2. — About four fifths the original size. Effects upon the strength of contractions of the anterior segments, produced by section of the conducting nerves. The nerve cord was cut at A, the right lateral nerve at B, and the left lateral nerve at C, thus stopping further contraction of these distal segments. Time = one fifth of a second.

of the myocardium innervated (Fig. 2). Further experiments revealed the cause of this diminution in the height of contraction, and showed that the muscle elements involved were those which were most directly innervated by the sectioned nerves. Thus upon section of a lateral nerve the chief effect is upon the homolateral half of the myocardium. This is shown in Fig. 3, which was taken from an experiment conducted in the following manner. The heart was fastened by pins to a paraffin block, weighted and submerged in sea water. The pins were inserted about the margin of the posterior seven segments. In the anterior two segments they were placed in the median line, just avoiding the central nerve cord. Tracings were then taken from the lateral halves of these anterior segments. When the left lateral nerve was cut (at X, Fig. 3), the contractions of the left side were at once diminished to about one sixth of the original height. After about eight minutes (at the point Z, Fig. 3) recovery had taken place and was almost complete.

Discussion. — In interpreting these findings we believe we are justified in postulating a predominant homolateral innervation of the myocardium of *Limulus*. Impulses passing along a given path affect more especially those cells which they innervate most directly, and in case the more direct path is interrupted then recovery and co-ordina-

¹⁰ CARLSON, A. J.: *Ergebnisse der Physiologie*, 1909, viii, p. 408, Fig. 15.

tion are effected by transmission of the impulses by a more indirect or circuitous route. Functional integrity of the myocardium is thus re-established either by conduction from muscle cell to muscle cell (which does not seem probable in the light of Carlson's¹¹ work) or by the establishment of new paths in the peripheral nerve plexus.¹² In this way function, lost by the section of the more direct paths, is re-established. The whole arrangement is strongly suggestive of the disposition of inhibitory nerve fibres found by Garrey¹³ in the heart of the turtle. In the turtle the action of the inhibitory nerves is more pronounced on the homolateral side of the heart—a bilateral effect is obtained with stronger stimuli. The view that a similar condition may exist in *Limulus* is strengthened by the results of stimulation of the nerves. After ablation of

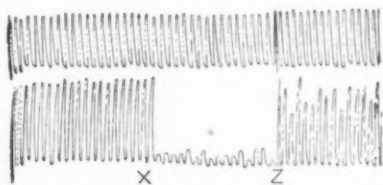


FIGURE 3.—Upper tracing from the right half and lower tracing from the left half of the second segment. The left lateral nerve was cut at X and produced only a homolateral effect. Note the recovery at Z. Heart's rate = 20 per minute.

the nerve cord from the posterior segments, a procedure which produced quiescence of the heart and prevented inhibitory phenomena, stimulation of a lateral nerve with very weak induced currents (either faradic or single) produced a much stronger contraction on the homolateral half of the anterior segments. Strong shocks produced a bilateral effect. These results are in perfect accord with those obtained upon the turtle's heart when a vagus nerve was stimulated. The effects of section of the nerves of *Limulus* heart as here outlined furthermore remind one forcefully of the experiments of

¹¹ CARLSON, A. J.: this Journal, 1907, xvii, pp. 478 *et seq.*

¹² Section of some of the fibres of a motor nerve paralyzes the fibres of skeletal muscles which they innervate. The contraction of the muscle as a whole is correspondingly weakened. It is somewhat surprising that a similar condition is to be found in the *Limulus*' myocardium, which is a syncytium. CARLSON has shown, however, that in spite of this anatomical condition conduction does not take place by transmission of the impulse directly from muscle element to muscle element, but, normally, is always by way of the nerve plexus. After treatment of the myocardium with sodium chloride (or other chemicals) a direct muscular conduction may be established.

¹³ GARREY, W. E.: this Journal, 1911, xxviii, p. 330.

Gaskell on the turtle's heart. He produced blocks of varying degrees by successive sections which narrowed the conducting bridge; similarly in our experiments the response of the muscle is also affected by decreasing the number of conducting nerve fibres, *i. e.*, the functioning area of cross section.

The results of section and stimulation of nerves, as we have outlined them above, justify the conclusion that the effects of clamping of the nerves may be due in part to destruction of function (temporary or permanent) of some of the compressed fibres, *i. e.*, by actually cutting off certain paths of conduction while others still function. By this procedure a corresponding portion of the myocardium contracts less efficiently. Many of the phenomena, however, cannot be ascribed to this mechanism, and are compatible only with the alternative view that they are produced by an alteration in the property of conduction in all the fibres.

After a clamp has been tightened on the nerve cord, say at the level of the junction of second and third segments, till the myocardium of the anterior segments fails to respond to the impulses from the posterior part of the nerve cord, recovery more or less complete, depending upon the severity of the compression, will take place (see Fig. 8). This fact alone speaks strongly for an involvement of all of the fibres in the altered conduction. Other facts which speak for this view will be considered in the subsequent sections.

THE RELATION OF STRENGTH OF THE IMPULSES TO BLOCK.

a. **Experimental variations in the strength of impulses.** — A degree of compression which will block a weak stimulus will not block a stronger stimulus; strong induction shocks, for example, applied to the lateral nerves posterior to the clamp after the normal impulses had been completely blocked, frequently caused contractions of the anterior segments. Strong autogenous neurogenic impulses may also break through a block which is complete for weaker impulses. One of the most striking methods of demonstrating this relation of strength of stimulus is to produce complete block by clamping the nerves, and to then subject the ganglionic nerve cord in its posterior portion to the action of stimulating chemicals, such as isotonic solution of chloride, citrate, or tartrate of sodium, which is added to the sea water or *Limulus*' blood

plasma in which the nerve cord is immersed. These chemicals increase the rate of discharge and the force of all impulses sent out by the ganglion. In all hearts thus treated, as soon as the impulses had reached a sufficient strength the previously existing block disappeared and the segments anterior to the clamp began to contract. This is illustrated in a somewhat typical way in Fig. 4, in which the nerve cord was transferred from sea water to isotonic sodium chloride at the point A. The

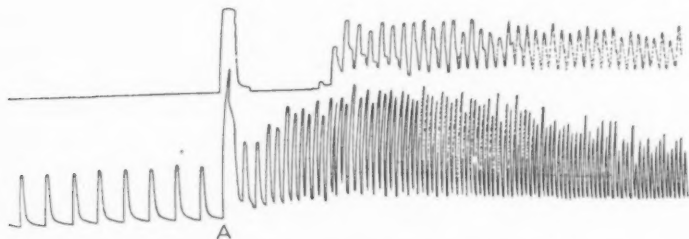


FIGURE 4. — Illustrates the disappearance of block upon stimulating the nerve cord (but not the site of compression) with 3 per cent sodium chloride at A. Also shows the refractory condition of the nerve due to clamping and activity, only alternate impulses producing full effects. Frequently the alternate impulses produce no effect, thus establishing the 2/1 rhythms of partial block. The smoked paper moved 24 millimetres per minute.

increased strength of impulses is recorded in the contractions of the posterior segments (lower tracing). Four seconds later the strengthened impulses forced the block, and the anterior segments contracted. In cases in which the increased strength of the impulses was sustained by the proper admixture of sea water and sodium chloride solution, it was found that the impulses continued to force their way through the compressed area. As soon, however, as the nerve cord was replaced in sea water and the impulses were thereby weakened, the condition of block was re-established. When the stronger impulses had forced a block, it was found that the block was easily re-established by increasing the amount of compression by tightening the clamp, and that the block again disappeared when the clamp was released. In perfect accord with these experiments are the results of Meek and Leaper,¹⁴ who found that strong artificial stimulation of a motor nerve would cause an impulse to pass a region of compression when weaker stimuli produced no

¹⁴ MEEK and LEAPER: *Loc. cit.*, p. 315.

effect. An examination of the published tracings of Ducceschi¹⁵ shows also that, as a result of compression of motor nerves, the effects of the weaker make induction shocks disappeared before those due to the stronger break shocks, although the author makes no reference to the finding.

b. **Partial block due to impulses of various strength.** — It was very commonly found that the impulses originating in the ganglion varied

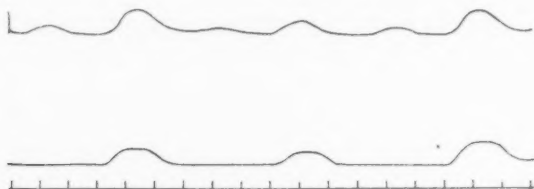


FIGURE 5. — A 2/1 rhythm established by blocking out the weaker of two unequal impulses, as seen in the upper tracing taken from the posterior segments. Lower tracing from anterior segments. The clamp was placed between the two. Time record indicates seconds.

in strength in a perfectly regular manner, for example, a strong impulse frequently alternated with a weak one. In other instances the impulses appeared in groups of three or four; the first of the group producing the strongest contraction, the last impulse producing the weakest contraction, while the intermediate contraction had a strength graded between the other two. In several such instances the clamp was applied and the conducting nerve paths compressed. There always resulted a gradual weakening of the contractions anterior to the point of compression after the manner already described. In every instance a stage ultimately was reached at which the weak impulses were blocked while the stronger impulses were still transmitted. Fig. 5 shows the results of one of these experiments. In this instance the nerve cord generated impulses alternately weak and strong, as is shown in the upper tracing. Clamping produced a 2/1 rhythm, since the weaker impulses were blocked and only the stronger caused contractions of the anterior segments (lower tracing).

The same condition is shown in a very striking way in Fig. 6. At A, B, and C the clamp was tightened but complete block was not produced. Near the middle of the record an alternating rhythm sud-

¹⁵ DUCCESCHI: *Loc. cit.*, Fig. 4, p. 44.

denly made its appearance.¹⁶ The weaker impulses failed to get through the compressed region, and a 2/1 rhythm was instituted. That the result was due purely to a diminution in the strength of the impulses is clearly demonstrated by the fact that whenever a strong impulse replaced a weak one, as at 1-1, 2-2, and 3-3, it was conducted through in the normal way and produced contraction. These examples indicate clearly that in any condition in which the impulses vary in strength, compressing the conducting paths may lead to the establishment of a partial block. It can be readily seen that the type of block will depend upon the grouping of the unequal impulses and the degree of compression. A strong impulse alternating with a weak one can give only a 2/1 rhythm. Other types of partial block are developed, however, in such groupings as are indicated in Fig. 7, which diagrammatically represents two types of grouping, tracings of which have been obtained in the course of this work. Slight compression represented by the line *a* eliminates the weakest contraction

¹⁶ The preparation had previously been subjected to the action of potassium permanganate and of calcium chloride solutions.

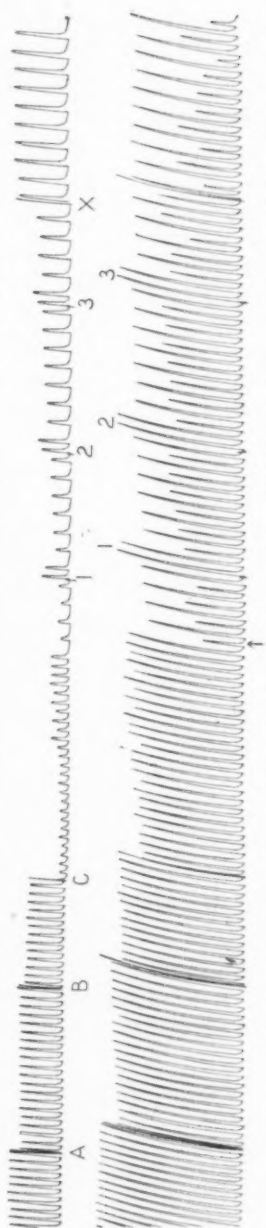


FIGURE 6.—Seven-ninths the original size. Effects of clamping shown at A, B, and C. With the development of impulses of alternating strength (7) the weaker fails to reach the anterior segments (upper tracing), thus establishing a 2/1 rhythm. When strong impulses replace the weaker, as at 1-1, 2-2, and 3-3, they overcome the block at the compressed area. At X a solution of disodium phosphate was added to the plasma about the anterior segments, and increased their irritability so that the weaker impulses produce a just perceptible contraction. The smoked paper moved 22 millimetres per minute.

(4) in both group *A* and group *B*. There thus is developed a partial block with a $4/3$ rhythm. Greater compression (represented by the line *b*) causes a $4/2$ block in group *A* and a $2/1$ block in group *B*. The amount of compression indicated by the line *c* develops a $4/1$ rhythm of partial block in both groups.

It is thus seen that a variety of different types of partial block may be developed as a result of inequalities in the strength of the impulses

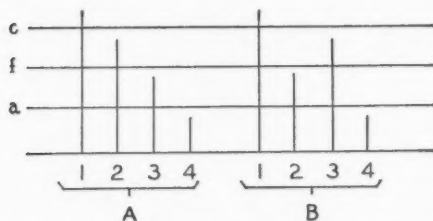


FIGURE 7. — Diagram representing two groups of four impulses (1, 2, 3, 4) showing some variations in strength of impulses frequently encountered in automatic tissues. The lines *a*, *b*, and *c*, established on the ordinates at different distances, represent different degrees of compression of conducting tissue. The portions of the ordinates above these lines represent the impulses which pass through the clamp and their relative strength. Compression *a* established a $4/3$ rhythm in both groups; compression *b* results in a $4/2$ rhythm in group *A* and a $2/1$ rhythm in group *B*; while compression *c* establishes a $4/1$ rhythm in both groups.

generated in the rhythm-producing tissue. The number of types of block which may be produced in this way is, however, limited for each grouping of the impulses. This fact places a distinct restriction upon the application of this mechanism to the partial blocks produced in the vertebrate heart by similar compression. Although it is conceivable that there may be distinct variations in the strength of the rhythmic impulses which develop in the vertebrate heart, and that such varia-

tions may cause certain types of block upon compression, yet such a factor ordinarily can serve only as a modifying influence upon the other mechanisms involved. This is clearly understood when we remember that both Gaskell and Erlanger have shown that it is possible to pass step by step through all the intermediate stages from the slightest degrees of partial block to the most complete block. This result cannot possibly be obtained in the case of partial blocks due to differences in the strength of the impulses such as have just been considered.

PARTIAL BLOCKS INDUCED BY THE PASSAGE OF IMPULSES THROUGH
THE COMPRESSED REGION.

In addition to the partial blocks produced by the coincidence of variations in the strength of impulses and the proper degree of compression, our experiments show that partial block may also be instituted by changes in the physiologic properties in the compressed portion of the nerves. The changes are due to the passage of the impulses themselves, and are of such a nature that the passage of one or more impulses through the compressed area renders the nerve incapable of conducting succeeding impulses effectively. These alterations in the properties of the nerve occur independently of any alteration in the strength of the ganglionic impulses or of any alteration in the excitability of the myocardium.

Indications of these changes are to be found in nearly every tracing. In their simplest form they appear as a distinct irregularity in the height of contraction of the anterior segments, distal to the point of compression. These irregularities are most noticeable in the severer grades of compression, when all impulses are much weakened, and in the interval following the release of the clamp subsequent to compression. The variations in the height of contraction may recur with distinct regularity. This is to be seen in Fig. 4. In this tracing it is to be noted that when the block was overcome the impulses affecting the anterior segments were alternately strong and weak. The weaker impulses are in evidence as a slight shoulder on the descending limb of the stronger contraction. When the ganglionic impulses became weaker, this shoulder disappeared because the weakened impulse could not pass the block. A $2/1$ rhythm of partial block was thus instituted. The experiment represented in Fig. 8 brought out very clearly the irregularities and partial blocks established by severe compression at *A*. Following decompression at *R*, recovery gradually ensued, but during this stage there is furnished a very striking instance of the type of partial block under consideration. It may be noted that at first there was a short stage in which every second impulse was ineffective. Following this it is to be noted the contractions appear in groups which are separated by intervals in which the ganglionic impulses failed to pass through the block. Toward the end of the tracing the block disappeared and the normal sequence of beats was re-established and

maintained. The condition is quite comparable to the stages of slight block which may be established in the vertebrate heart by clamping the auriculo-ventricular junction. That the groups in the blocks of the vertebrate heart appear more regularly is only a relative matter. They may appear irregularly in vertebrates and occasionally with perfect regularity in the heart of *Limulus*. There is then justification for believing that this mechanism is applicable to the explanation of

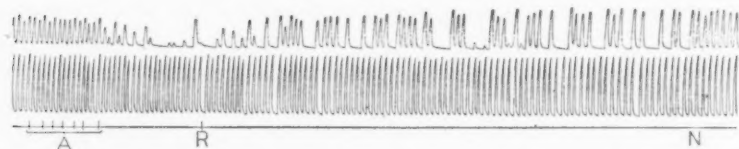


FIGURE 8. — Shows partial block with groups of contractions separated (irregularly) by dropped beats. Effects of clamping at *A*, clamp released at *R*, and normal sequence re-established at *N*. Upper tracing is from the second segment, the lower from the sixth segment — clamped between the second and third segments. The smoked paper moved 22 millimetres per minute. About one half the original size.

partial block as it appears in the vertebrate. The tracings of Ducceschi¹⁷ show that compression of a motor nerve transforms a complete tetanus of the muscle into an incomplete tetanus, and also show that the impulses which pass through the area of compression vary greatly in strength and number. These results harmonize with our findings as outlined above. Our experiments, however, show how important this factor of lengthened refractory period or fatigue, if we may so designate it, really is in the explanation of the mechanisms of partial block.

a. Condition of the nerve which results in this type of block. — The condition which results from compression of a nerve is one of general sluggishness. The ability of the nerve to conduct impulses is distinctly diminished, and while conclusive proof has not been adduced to show that conduction time is lengthened, yet there are grounds for believing this to be the case. Any procedure which tends to increase the excitability and conductivity of the compressed nerve may cause the irregularities which have just been considered to disappear. This happened, for example, in the experiment from which Fig. 4 was taken. When sodium chloride was applied at the site of clamping, the partial block disappeared at once and every impulse passed readily through the clamp.

¹⁷ DUCCESCHI: *Loc. cit.*, p. 49, Figs. 6 and 7.

Attention is again directed to the obvious fact that although one or more impulses may pass the clamp they leave the nerve in such a condition that one or more succeeding impulses are conducted with difficulty. They are weakened or rendered ineffective by the physiological activity of the nerve. This condition of the nerve remains until a certain, although somewhat variable, time interval has elapsed. We may look upon this interval as a prolonged refractory period, not unlike that produced in nerves by Fröhlich¹⁸ through the agency of asphyxia and anaesthetics. To test this idea, a condition of partial block was produced by clamping. The ganglion was then cooled, while the temperature of the clamped portion of the nerves remained constant. Cooling from 28° C. to 20° C. produced a slowing of the heart rate from 16 per minute to 9 per minute. The effect of this procedure upon the strength of the impulses, as recorded by the posterior segments, was almost imperceptible, but the condition of partial block immediately disappeared. The impulses all passed to the anterior segments and produced contractions when the rate was slow. When the ganglion was again warmed to 28° C., the condition of block again supervened.

b. Discussion. — It is our belief that the result of cooling the ganglion was due wholly to the slower rate which gave ample time between impulses for the nerve to recover from the abnormal state occasioned by their transmission. It is a tempting field of speculation to attribute the phenomenon of block, which is produced by the conduction of impulses, to a condition similar to Wedensky's¹⁹ "parabiosis"; a condition which Semenoff²⁰ has shown may be produced by compression of nerves. The applicability of this explanation is, however, rendered highly improbable by the work of Hoffmann,²¹ who has demonstrated with the string galvanometer that each contraction of the myocardium of *Limulus* is not a single twitch, but a tetanus. We cannot be more specific than to state that we believe the blocks are due to a refractory condition of the nerve which is the result of fatigue. Fatigue has been demonstrated by Meek and Leaper²² in nerves which have been sub-

¹⁸ FRÖHLICH, F. W.: *Zeitschrift für allgemeine Physiologie*, 1904, iii, pp. 474 *et seq.*

¹⁹ WEDENSKY: *Archiv für die gesamte Physiologie*, 1903, c, p. 182.

²⁰ SEMENOFF: *Ibid.*, 1903, c, pp. 1-144.

²¹ HOFFMANN (PAUL): *Archiv für Physiologie*, 1911, pp. 142 *et seq.*

²² MEEK and LEAPER: *Loc. cit.*, p. 318.

jected to pressure; fatigue of nerves is in fact a general accompaniment of general sluggishness such as may be produced by cold (Tait²³), by anæsthetics (Fröhlich²⁴) and by yohimbin lactate (Tait and Gunn²⁵).

THE RELATION OF DEPRESSED EXCITABILITY OF THE MYOCARDIUM TO HEART BLOCK.

It has developed regularly in the course of our experiments that clamping of the nerves weakened the impulses to a point where the myocardium failed to give a mechanical response. In many such instances it was found that if the excitability of the muscle was increased, contractions were again obtained. This result was produced in individual cases by warming the myocardium or by adding small quantities of isotonic solutions of sodium chloride, citrate, tartrate, or dibasic phosphate to the sea water in which it was immersed. An indication of this result is to be seen in Fig. 6. At X 5 c.c. of n/2 disodium phosphate were added to the 50 c.c. of sea water in which the anterior segments were immersed. The increased excitability is shown in the increased height of contractions, and careful inspection of the tracing shows that there are, interpolated between the higher contractions, very small contractions which were caused by the weaker impulses from the nerve cord, contractions which were recorded in spite of the fact that the impulses giving rise to them were actually weaker than in the preceding portions of the tracing, where they produced no effect. That a partial block existed in this experiment was due then not only to alternating strength of impulses, but also to the fact that the myocardium was not excitable enough to respond to the weakened impulses. There can be no question, then, that impulses may actually pass the clamp without being effective, nor that the effects which are produced by impulses which reach the muscle through the clamped region are determined in a certain measure by the physiologic condition of the musculature.

An illustration of a peculiar but not infrequent response of the

²³ TAIT: Quarterly journal of experimental physiology, 1908, i, pp. 92 *et seq.*

²⁴ FRÖHLICH: *Loc. cit.*, p. 478.

²⁵ TAIT and GUNN: Quarterly journal of experimental physiology, 1908, i, pp. 191 *et seq.*

muscles of *Limulus* heart is shown in Fig. 9. The contraction of certain segments appear in groups in which the individual contractions get progressively weaker (lower tracing), although the impulses which caused these contractions remain fairly constant (upper tracing), or at least do not vary uniformly with the grouping.²⁶ It is apparent that a uniform decrease in the strength of all the impulses, such as would result from clamping the nerves, would lead to a dropping out of the

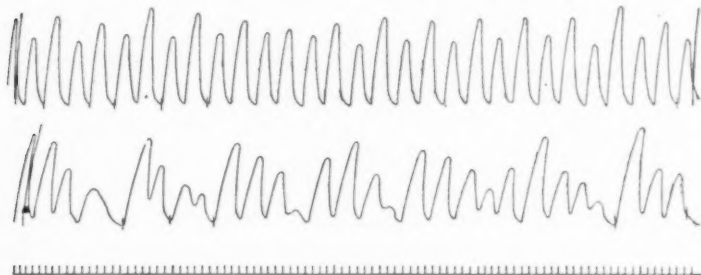


FIGURE 9. — Owing to treatment with chemicals, the contractions of the myocardium of the posterior segments (lower tracing) are not commensurate with the strength of impulses as indexed by the contractions of the anterior segments (upper tracing). Clamping conducting tissue in such a condition of the myocardium would establish partial blocks even if the impulses were of equal strength.

weakest contractions of each group and that a condition of partial block would thereby be produced. This was tested by an actual clamping experiment and the expected result obtained; a condition of partial block appeared.

GENERAL APPLICATION TO HEART BLOCK OF THE VERTEBRATE HEART.

Our work on the nerves of *Limulus* heart has given detailed information concerning the actual factors which must be considered in any explanation of heart block due to compression of the conducting system. It remains for us to see how well they fit into the explanation of heart block in the vertebrate heart. The striking similarity of our results to those obtained on vertebrate hearts leads us to believe that

²⁶ This condition of the muscle was induced by treating successively with a number of solutions; the actual cause of the peculiarity of this muscle is, however, not known to us.

the same factors are involved when all types of conducting tissue are compressed, and that the differences which do appear can readily be accounted for when we consider that the myocardium of *Limulus* acts like skeletal muscle, while the vertebrate contracts according to the "all or none" rule, and possesses a refractory period of a distinctive character. That the conducting paths are nerves in the cases we have considered above, and that they probably are not in the vertebrate heart, we feel certain makes little, if any, difference in the general application of our findings.

In applying our results we turned at once to the admirable schematic representation of the mechanism of heart block due to clamping the His' bundle which Erlanger²⁷ has given us. This investigator lays special emphasis upon two factors as the probable causes of the partial blocks which he observed. One of them is the gradual and progressive weakening of the impulses which pass the clamp when it is gradually tightened; the other is the variation in the excitability of the ventricular musculature subsequent to contraction. Our experimental results harmonize perfectly with this conception of the mechanisms involved; compression weakens the impulses transmitted, and the effects produced by these impulses depend upon the excitability of the musculature.

Our experiments show that at least two additional factors must be considered in individual cases of heart block. The first of these is the not infrequent variations in the strength of the impulses which originate in the rhythmic tissues. While this is undoubtedly much more a factor in the neurogenic heart of *Limulus* than in the vertebrate heart, still it occasionally becomes a factor in the latter. It is a not infrequent occurrence to find cases in which the vertebrate auricles beat, first weakly then strongly, with rhythmic variability. Clamping in such instances could result in a block of a fixed type. The limitations of this factor as a mechanism of importance are, however, apparent, and have already been referred to in the discussion of our experiments.

The other factor to which we wish to direct attention, one which has not been found by previous investigation, is the change in the conductivity of the compressed tissue which is brought about by the

²⁷ ERLANGER, J.: this Journal, 1906, xvi, p. 183, and American journal of medical sciences, 1908, cxxxv, p. 797.

passage of impulses; as shown above, the tissue is thus fatigued or rendered refractory, so that succeeding impulses are blocked until recovery has occurred. This factor should not be overlooked in the application of Erlanger's mechanism, for it will serve in the explanation of certain irregularities which are frequently encountered in the grouping of contractions in partial block.

Erlanger's diagram makes it clear that in partial block, experimentally produced, many impulses actually pass the clamp, but fail to produce any effect because of their reduced strength and the reduced excitability of the ventricular musculature subsequent to its contraction. This fact is not commonly recognized and has never been put to the experimental test. Our experiments, however, show that the condition actually existed in some of our cases, that impulses which were completely blocked, so far as any contraction of the myocardium was concerned, actually passed the compressed area but were below the threshold for the muscle. When the excitability of the latter was raised by appropriate treatment with chemicals, these stimuli became effective.

SUMMARY.

1. Progressively increasing the compression upon the nerves of *Limulus* heart causes a progressive reduction in the height of contraction of the myocardium supplied by the corresponding nerves; the full effects do not come on immediately, but gradually increase with a constant pressure.

2. Recovery may take place after releasing from compression.

3. Cutting, clamping, or stimulating the cardiac nerves causes changes in height of myocardial contraction which are mainly homolateral in the case of lateral nerves, but bilateral in the case of the nerve cord. After cutting the lateral nerves the decreased height of contraction may be recovered from, probably as a result of impulses which pass by new or more circuitous paths.

4. Clamping nerves may block out weak impulses, while stronger impulses are only weakened; thus variations in the strength of nerve impulses may give rise to various types of partial block depending upon the degree of compression of the conducting paths.

5. Compression establishes in nerves a condition as a result of

which the passage of one or more impulses renders it refractory to a succeeding impulse, thus accounting for certain conditions of partial block.

6. Impulses may pass a clamped region and yet be below the threshold of the myocardium, or they may find it in a refractory state; thus in certain cases the condition of the myocardium may be the factor determining the type of partial block established by clamping the nerves.

REACTIONS OF THE VASOMOTOR CENTRE TO SECTION AND STIMULATION OF THE VAGUS NERVES.

BY TORALD SOLLMANN AND J. D. PILCHER.

[From the Pharmacological Laboratory of the Medical School of Western Reserve University,
Cleveland, Ohio.]

THE following investigations have been made by the method described in our previous paper;¹ namely, by the artificial perfusion of an organ in a living animal, with the nervous connections intact, but completely separated from the circulation of the animal. With this arrangement sudden changes in the perfusion flow can be produced only through the vasomotor centre; a quickening of the flow indicating vasodilation, and a slowing, vasoconstriction, always of central origin. The study of the vagus effects appeared doubly important, because they are apt to occur incidentally in other conditions which may be investigated.

DIVISION OF BOTH VAGI.²

This causes almost invariably a moderate stimulation of the vasomotor centre, involving the vessels of the kidney as well as those of the spleen, both in dogs and in cats (Fig. 1).

In animals with natural respiration the stimulation of the vasomotor centre could be explained by partial asphyxia, from the slowed respiration which normally follows division of the vagi. This, in fact, explains the stimulation in part, but apparently not altogether, for in animals under artificial respiration (either oxygen insufflation or bellows), section of the vagi still causes unmistakable vasoconstriction, although less than in animals breathing naturally. This may be seen in Table I.

¹ SOLLMANN and PILCHER: this Journal, 1910, xxvi, pp. 233-238.

² In the neck.

With natural respiration section of both vagi produced recognizable vasoconstriction in thirteen out of sixteen dogs (81 per cent) and in three cats (100 per cent). With artificial respiration vasoconstriction was recognizable in five out of eight dogs (63 per cent), and in six out of nine cats (67 per cent).

It will again be noted that the vasoconstriction is much more constant with natural respiration.

The behavior of the vasomotor centre accords well with the behavior of the blood pressure (see Table II). When both vagi are divided, there is commonly a rise of blood pressure which in morphinized dogs (under light ether anesthesia) averages about 25 mm. This is partly attributable to the quickening of the heart rate, which in these dogs amounts to nearly 60 per cent; but it is not explainable altogether by this, for in curarized dogs, in which the vagus tone is already depressed (so that the quickening of the heart amounts to only 28 per cent), the average rise of pressure is still 20 mm.; and it rises 12 mm. even in atropinized cats, in which the section of the vagi cannot alter the heart rate.

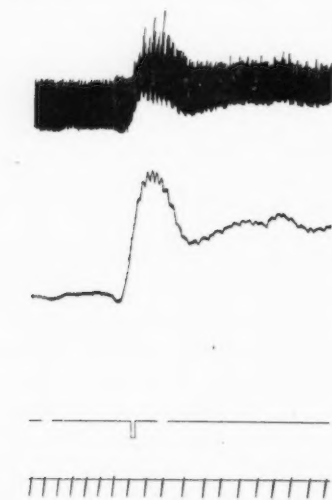


FIGURE 1. — Division of both vagi (Dog C 82-1). The upper tracing is the carotid pressure taken with a membrane manometer. The second tracing is from a damped mercury manometer. The lowest line shows the slowing of perfusion flow through the kidney. The vagi were divided at the signal on the abscissa. The heart rate was 136 per minute before division and 180 after division.

It is therefore necessary to assume a vasoconstriction, and this again cannot be abolished by artificial respiration, for atropinized cats with artificial respiration still show a rise of blood pressure, often small, it is true, averaging only 6 mm.; but sometimes 15 mm. and fairly constant. Pawlow³ found that curarized and atropinized dogs also give a rise, averaging 15 to 25 mm.

The vasoconstriction can also be deduced from the cardiac volume. As the vagi are divided and the blood pressure rises, cardiac plethysmo-

³ J. PAWLOW: Archiv für die gesammte Physiologie, 1879, xx, p. 210.

grams (Experiment C 180 to 189) generally show temporary cardiac dilation with diminished excursions (Fig. 2). The dilation indicates

TABLE I.

Slowing of vein flow from prepared organs on division of both vagi. These figures show the percentage of slowing, when the rate of flow just prior to division is compared with the flow three to five minutes after division. They are the averages of the number of experiments which is indicated in the parentheses. The greater the slowing, the greater is the stimulation of the vasoconstrictor centre.

Animals with natural respiration.	Animals with artificial respiration.
Dogs' spleen 20 per cent (12 expts.)	8 per cent (7 expts.)
Dogs' kidney 20 per cent (4 expts.)	11 per cent (2 expts.)
Cats' spleen 35 per cent (2 expts.)	10 per cent (8 expts.)
Cats' kidney 17 per cent (1 expt.)	8 per cent (2 expts.)

that the heart is throwing out less blood, notwithstanding the faster rate, until it becomes adjusted to the new conditions. Asphyxia was excluded by artificial respiration.

TABLE II.

Changes in blood pressure and heart rate following division of both vagi. The readings are taken about three to five minutes after the division; the number of animals which were used in calculating these averages is stated in the parentheses.

	Changes in heart rate (per minute).	Changes of blood pressure (mm.).
Morphinized dogs with natural respiration.	{ 98-156.5 = 58.5 (13 expts.)	114-139 = 25 (17 expts.)
Morphinized and curarized dog with artificial respiration.	{ 116.5-149.5 = 33 (8 expts.)	110-130 = 20 (9 expts.)
Cats with atropin-morphin-ethylcarbamate anesthesia.	{	140-152 = 12 (3 expts.)
Cats, same anesthetic, with artificial respiration.	{ 212-220 = 8 (2 expts.)	127-133 = 6 (11 expts.)

Since the heart is working less efficiently at this stage, the rise of blood pressure must be due to vasoconstriction.

We have not attempted to analyze this vasoconstrictor action further. It is known that the vagus carries afferent constrictor fibres (see the next section), and it is conceivable that these are stimulated by the trauma. In harmony with this, it is often observed that the pressure rises higher immediately after division, when the traumatic irritation is strongest. This extra rise usually disappears in a few

moments; but the moderate rise, and the stimulation of the vasomotor centre which we have studied persist for at least five minutes.

The division of the depressor fibres might be invoked as a partial explanation of the rise (Pawlow³), especially for cats; but this is improbable, since there is no conclusive evidence that the depressors are tonically active.

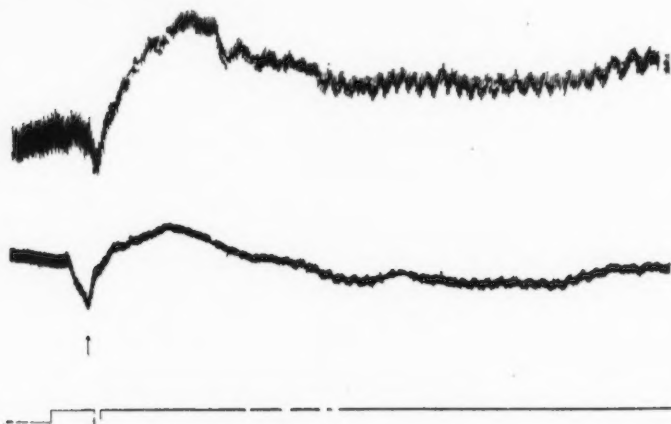


FIGURE 2.—Effect of division of vagi on heart and blood pressure: Upper tracing, cardiac plethysmogram (rise corresponds to increased volume), second tracing, carotid blood pressure. The vagi were divided at the arrow; the preliminary fall is due to traction. The heart rate increased from 138 to 173 per minute (Morphinized Dog C 181; no curare).

STIMULATION OF THE CENTRAL STUMP OF THE VAGUS IN DOGS.

Both vagi were divided in the neck in curarized dogs. The results of stimulation are illustrated by Fig. 3 and by the following data:

Experiment.	Blood pressure.	Spleen flow.
C 17-1	125-146 = 21 mm.	33-22 = 33 per cent.
C 27-1	130-167 = 37 mm.	35-33 = 6 per cent.

The flow is also slowed when the intact vagi are stimulated (Dog C 20-1).

This vasoconstriction on afferent vagus stimulation is entirely analogous with that of sciatic stimulation. When the two are stimulated

on the same animal, the response is nearly quantitatively identical (Fig. 3). Furthermore, when the blood pressure was raised by vagus stimulation, sciatic stimulation did not produce any further rise (Experiment 27).

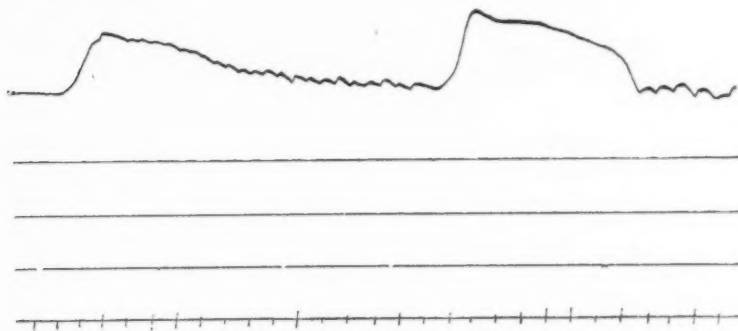


FIGURE 3. — Stimulation of the central stump of the sciatic to the left and of the vagus to the right. The lowest line shows the slowing of the spleen flow (Curarized Dog, C 27-1).

None of our dogs showed any depressor reaction on vagus stimulation. This agrees with the experience of Seelig and Lyon,⁴ who emphasize the "remarkably constant pressor effect" elicited from this nerve, which they find preserved even into deep shock. Bayliss⁵ states that pressor response is the common effect, but that depressor response is often obtained after prolonged operations. Of this we have had no experience. The statement of Howell⁶ that "a depressor effect is usually obtained" would seem to be an error.

The very different reaction of cats, in which the vagus usually carries depressor fibres, will be discussed in our next paper.

STIMULATION OF THE PERIPHERAL END OF THE VAGUS.⁷

This was tried on five dogs with spleen perfusion. Curare was not used. It was found that the heart escapes from the vagus control too

⁴ M. G. SEELIG and E. P. LYON: *Surgery, gynecology, and obstetrics*, 1910, p. 146.

⁵ W. M. BAYLISS: *Journal of physiology*, 1902, xxviii, p. 287.

⁶ H. H. HOWELL: *Text-book of physiology*, 2d ed., p. 563.

⁷ In the neck.

quickly to give entirely satisfactory results by our method. In two experiments in which the heart was merely slowed, the spleen responded by undoubted constriction (Fig. 4).

The three experiments in which the heart was arrested, showed a brief but marked quickening of the vein flow which was followed by

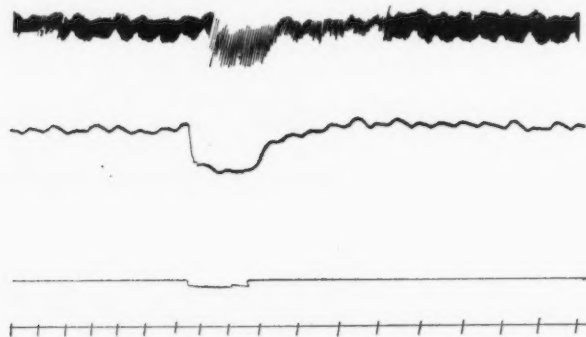


FIGURE 4. — Peripheral vagus stimulation. (Dog C 31-1). Upper tracing, membrane manometer, second tracing, damped mercury manometer in carotid artery. Third tracing, duration of stimulation. Lowest tracing, spleen flow.

slowing. On account of the quick sequence it is difficult to tell whether the quickening was due to dilation or merely to the expression of blood from a powerful constriction. We incline to the latter view, and would attribute the whole effect to acute cerebral anemia. The pulse pressure showed increased excursion, the diastolic pressure falling more than the systolic pressure.

CONCLUSIONS.

1. *Division of both vagi* stimulates the vasoconstrictor centre, partly by the interference with respiration, and partly by traumatic irritation of the afferent vasoconstrictor fibres in the nerve. This is largely responsible for the rise of blood pressure.

2. *Electric stimulation of the afferent vagus* in dogs produces vasoconstriction, resembling quantitatively the response to afferent sciatic stimulation.

3. *Stimulation of the efferent vagus* produces results which may be interpreted as vasoconstriction, referable to acute cerebral anemia.



THE CONDITION OF THE DIGESTIVE TRACT IN PARATHYROID TETANY IN CATS AND DOGS.

By A. J. CARLSON.

[From the Hull Physiological Laboratory of the University of Chicago.]

THE parathyroid tetany syndrome in dogs comprises a number of symptoms that point to involvement of the digestive tract. These are salivation, anorexia, vomiting, and the usual gastro-enteritis. It is not known which of these, if any, are primary factors in, and which are secondary effects of the tetany. On the theory that the hyperexcitability of the cerebrospinal axis and the skeletal musculature in this tetany is due to the action of a circulating toxin or toxins, these toxins may have similar actions on the sympathetic and the autonomic nervous systems. This would result either in inhibition of secretory or motor activity, in hyperactivity, or in paralysis through failure of normal co-ordination. Deficient secretion of the digestive glands and paralysis of the neuro-muscular mechanisms of the digestive tract would necessarily lead to anorexia and possibly vomiting. Our hypothetical tetany toxins may also act on the digestive glands directly either in the way of stimulation or depression. In dogs the tetany is usually accompanied by signs of pain and distress, and it is well known that pain tends to inhibit the activities of the digestive tract in the normal animal. On the other hand, the neuro-muscular apparatus of the digestive tract itself may exhibit the same degree of resistance to the "tetany toxins" as the heart tissues. In the dog and the cat the heart activity is not greatly altered in parathyroid tetany. There is usually some increase in the rate and strength of the heart beat in strong tetany, but this may be a secondary effect of the increased muscular activity similar to that in exercise. There is no inco-ordination or fibrillation similar to that in the skeletal muscles. The heart invariably persists in strong rhythm for several minutes after paralysis of the respiratory movements in death in an epileptic

attack. The changes in the pulse that accompanies tetany are probably due to the condition of the central nervous system and the muscular activity rather than to changes in the heart tissues, or hyper-tonus of the heart nerves.

METHODS.

1. Because of the usual persistent vomiting of dogs in tetany the X-ray method of recording the motor activities of the digestive tract after feeding bismuth mixtures did not seem feasible. Two devices were employed with more or less success to render the X-ray method available for the empty digestive tract.¹ Three loops of metal wire (platinum, silver, lead) were sewed on to the wall of the stomach and the intestines (the fundus, the antrum, the duodenum) in such a way as to form a triangle, the size of the triangle indicating the size of the lumen of the gut, and hence the degree of tonus or active contractions of the musculature. These wires become encapsulated within a few days and seem to cause little or no inconvenience to the dogs. The method was less satisfactory in cats, for most of the cats operated on in this way showed a poor appetite and occasional vomiting. A size of wire was selected that gave a distinct shadow on the fluorescent screen.

One centimetre long sections of catgut impregnated with silver nitrate and reduced with pyrogallic acid were secured to the walls of the stomach and intestines in the same way as the metal wires. Catgut thus treated is nearly as opaque as metal, it is not absorbed (at least not for four weeks), and causes less adhesions and inconveniences to the animals. But these devices, at best, give an imperfect view of small regions of the digestive tract, and after it was discovered that vomiting is the exceptional thing in parathyroid tetany in cats, these devices were abandoned for the bismuth mixtures.

2. One part of the work aimed at studying the motor activities of the digestive tract, if possible, independent of the secretion of the digestive fluids. For this purpose I used a mixture of 5 per cent solution of boiled starch, 1 per cent commercial peptone, 0.5 per cent hydrochloric acid, and 10 per cent subnitrate of bismuth. When warmed to 35°-40° C. this mixture is sufficiently fluid to be given

¹ CARLSON: Proceedings of the American Physiological Society, 1911, this Journal, 1912, xxix, p. xxviii.

through a stomach tube suited to cats. The 5 per cent starch solution, boiled for twenty minutes and acidulated, is a satisfactory carrier for the bismuth. The peptone was added to the mixture to insure the chemical conditions for gastric secretion. Appetite or psychic secretion is in all probability eliminated by the lack of appetite. A few experiments were made using the above mass not acidulated. The mixture was warmed to body temperature, thoroughly shaken, and given per stomach tube in quantities of 30 c.c. per kilo body weight of cat.

It would seem that the course of this mixture in the stomach ought to reveal the motor conditions of the stomach independent of gastric digestion. There is nothing in the mass that needs to be or can be digested in the cat's stomach. The hydrochloric acid is there to insure proper co-ordination of the pylorus. So far as can be determined there is nothing in the mass either to accelerate or to retard the gastric movements.

3. The time of retention of definite quantities of meat in the stomach involves the two factors of (1) secretion and of (2) movements. Thus meat feeding in tetany serves as an indirect method of studying gastric secretion in tetany. Our standard meat mass consisted of 10 gm. raw lean meat per kilo body weight of animal. The meat was passed three times through a meat grinder and 1 gm. peptone and 10 per cent bismuth subnitrate were added. These constituents were thoroughly mixed, but no water added, because all the tetany cats had to be fed forcibly, and it is much easier to force-feed the solid mass. Small masses of the mixture were placed on the back part of the tongue and manipulated until swallowed. Some of the control animals eat the mixture voluntarily, others had to be fed forcibly in the same way as the tetany cats. The peptone was added to the mass to minimize the influence of the varying factor of appetite secretion in the tetany group and in the control group. In the case of all the animals that had to be fed forcibly 5 c.c. of water was given at the end of the feeding to equalize the varying factor of salivary secretion.

4. In the experiments on dogs the starch-bismuth and the meat-bismuth mixtures were given in the same quantity per kilo body weight as that fed to the cats.

5. The time required for complete emptying of the stomach and of the small intestine was taken as a simple and reliable indicator of the

neuro-muscular efficiency of the digestive tract. When the meat mixture was used, accurate figures were usually obtained for the stomach only. In the case of the experiments with the starch-bismuth mixture observations were also made on the number and the rate of propagation of the antrum and the intestinal waves of contraction, and of the first entrance of the mass into the duodenum. It was hoped by this later point to determine the tonus condition of the pylorus in parathyroid tetany. It goes without saying that careful lookout was kept for actual tetany or contractures and spasms analogous to those of the skeletal muscles in tetany.

The frequency of the fluoroscopic inspection varied according to the points and measures involved. When it was desired to record only the total time of the starch-bismuth mass in the stomach and in the small intestine, the first observation was made one hour after feeding and then every half hour till the stomach was empty. The progress in the small intestine was then followed each hour, or each half hour, as the varying rapidity of the movements demanded. When the meat mass was fed, the first observation was usually made four hours after the feeding, and then every hour or half hour, depending on the mass remaining in the stomach. As to the time of entrance of the stomach mass into the duodenum in some cases the feeding was made directly under the fluoroscope, in other cases the observations were taken within five or ten minutes after the feeding.

The animals were not tied down for the fluoroscopic observations, but gently held in place and restrained by hand.

6. No attempts were made to remove the parathyroid glands while leaving the thyroids intact. Our parathyroidectomy is therefore equivalent to thyroid-parathyroidectomy. In the group of experiments involving section of the splanchnic nerves this operation was effected through an incision in the *linea alba*.

7. The galvanic excitability of the spinal nerves was measured by minimum strength of current required for the cathodic closing contraction when applied to the femoral nerve.

RESULTS.

I. The gastric and intestinal movements in parathyroid tetany as determined by the introduction of artificial chyme into the stomach. 1. Controls on normal

cats. — Fourteen feedings with the bismuth-starch mixture were made on seven normal cats. In six of the cats the time required for the mass to leave the stomach and the small intestines was remarkably uniform. The time in the stomach varied from ninety minutes to one hundred and thirty-five minutes, with an average of one hundred and twenty minutes, or two hours. The time for the small intestines varied from four and one-half hours to six hours with an average of five and one-half hours. The cat (No. XVI) not included in this summary showed in two experiments a delay of four hours in the stomach and of more than nine hours in the small intestine. In the case of the stomach this is more than double the average time of the other six cats, and this experiment must therefore be discarded. The cat was to all appearances normal. It is possible that the delay was due to masses of straw or hair in the stomach. Later in the course of the work it was found that masses of straw or boluses of matted hair occasionally present in the stomach of cats greatly delay the emptying of the stomach after feeding the starch mass. But our control Cat No. XVI passed out of observation before this possibility was recognized.

The time elapsing between the giving of the acid bismuth-starch mass by the stomach tube and the first appearance of the mass in the duodenum was more variable. In two cases it was less than five minutes. In four cases it was between five and ten minutes, while in the remaining feedings the time was between ten and fifteen minutes. In Cat No. XVI none of the mass passed out of the stomach during the first twenty-five minutes.

2. *Controls on normal dogs.* — Ten feedings of the bismuth-starch mixture to five normal dogs were made. The dogs selected were small (3.5 K. to 4.5 K.) in order to facilitate the X-ray observations. Accurate observations were made only on the time for the stomach. This was found to vary from three hours to five and one-half hours with an average of four hours. The dogs were healthy, vigorous, and to all appearances normal. The results indicate that under similar conditions the gastric movements in cats are more vigorous than in dogs.

3. *Cats in various stages of parathyroid tetany.* — Twenty-eight feedings of the bismuth-starch mass were made on sixteen cats in various stages of the tetany. The conditions include galvanic hyper-

excitability with or without tremors or salivation; strong tetany; galvanic hyperexcitability with depression and with subnormal body temperature. The results are somewhat variable, and cannot be adequately conveyed in a brief summary. A few typical experiments are therefore given in detail.

Cat No. III.

- Dec. 12, 10.00 A. M. Complete thyroidectomy.
 Dec. 13, 10.00 A. M. Animal in good condition, but does not eat.
 3.00 P. M. Labored respiration; tremors.
 3.10 P. M. 100 c.c. acid bismuth-starch mixture given by tube.
 4.45 P. M. Stomach empty.
 7.30 P. M. All the mass in the large intestine.
 Dec. 14, 8.00 A. M. Strong tetany; salivation; depression.
 2.00 P. M. Strong tetany; 100 c.c. acid bismuth-starch mixture given by tube.
 3.00 P. M. No gastric movements so far; all mass in stomach; tonus of stomach normal.
 4.00 P. M. Some of the mass in small intestine; strong tetany.
 7.00 P. M. Died in tetany. Post-mortem examination showed most of the mass still in the stomach and unchanged in consistency. Duodenum and upper jejunum filled with a mass concentrated to a solid continuous rod of unchanged starch-bismuth. Large intestine filled by the mass of April 13, much of the starch being unchanged.

Cat No. VIII.

- Jan. 23, 1912, 3.50 P. M. Cat normal. 100 c.c. acid bismuth-starch mass by tube.
 5.20 P. M. Stomach empty.
 8.50 P. M. All of the mass in the large intestine.
 Jan. 24, 4.00 P. M. 100 c.c. acid bismuth-starch mass by tube.
 4.10 P. M. Some of the mass in duodenum.
 5.20 P. M. Stomach empty.
 9.00 P. M. Nearly all of the mass in large intestine.
 Jan. 25, 11.00 A. M. Complete thyroidectomy.
 4.00 P. M. Condition good. C. C. C. = 1.7 M. A.
 Jan. 26, 8.00 A. M. C. C. C. = 0.5 M. A. No tremors.
 11.50 A. M. 100 c.c. acid bismuth-starch mass by tube.
 11.54 A. M. Some of the mass in duodenum.

- Jan. 26, 1.25 P. M. Stomach empty.
5.00 P. M. All of the mass in large intestine.
Jan. 27, 9.00 A. M. C. C. C. = 0.5 M. A.; some tremors; no feeding.
Jan. 28, 10.00 A. M. C. C. C. = 0.2 M. A.
12.10 P. M. 100 c.c. acid bismuth-starch mass by tube.
12.12 P. M. Some of the mass in the duodenum.
2.45 P. M. Stomach empty.
5.30 P. M. All of the mass in large intestine.
8.00 P. M. Died in tetany. Much unchanged starch in the contents of the large intestine.

Cat No. XIV.

- Jan. 29, 1912. Complete thyroidectomy and section of both cervical sympathetic nerves in the neck.
Jan. 30, 2.30 P. M. C. C. C. = 0.2 M. A.; no tremors or salivation.
3.00 P. M. 100 c.c. neutral bismuth-starch mass by tube.
3.04 P. M. Some of the mass in the duodenum.
3.30 P. M. Number of antrum waves per minute = 6. Time of passage of antrum waves, 34 seconds.
5.15 P. M. Stomach empty.
Jan. 31, 2.00 P. M. C. C. C. = 0.15 M. A.; no tremors or salivation.
3.00 P. M. 100 c.c. acid bismuth-starch mass by tube.
3.11 P. M. Considerable of the mass in duodenum; number of antrum waves per minute = 6.
5.00 P. M. Stomach practically empty; some tremors.
9.00 P. M. All of the mass in the large intestine.
Feb. 1, 8.00 A. M. Convulsions and salivation.
8.40 A. M. 100 neutral bismuth-starch mass by tube.
8.50 A. M. All mass yet in stomach.
10.00 A. M. Stomach empty.
1.00 P. M. Practically all the mass in the large intestine.
8.00 P. M. Violent tetany and salivation; no contractions of the large intestine during the attacks.
Feb. 2, 9.00 A. M. C. C. C. = 0.1 M. A.; tremors and depression.
3.00 P. M. 100 c.c. acid bismuth-starch mass by tube.
3.50 P. M. Antrum and intestinal movements normal.
4.30 P. M. Stomach empty.
7.30 P. M. All mass in large intestine.
Feb. 3, 8.00 A. M. Depression; temperature 37° C.
9.20 A. M. 100 c.c. neutral bismuth-starch mass by tube.
9.30 A. M. Some of the mass in duodenum.
10.50 A. M. Stomach practically empty.

Feb. 3, 12.20 P. M. Killed with ether. Some of the mass in lower ileum; part in the large intestine; abundance of unchanged starch.

Cat. No. X.

Jan. 27, 1912. C. C. C. = 1.5 M. A. Complete thyroidectomy.
 Jan. 28, 11.00 A. M. C. C. C. = 0.2 M. A.; no tremors.
 12.30 P. M. Mild tetany.
 3.55 P. M. 100 c.c. acid bismuth-starch mass by tube.
 4.00 P. M. Small amount of mass in duodenum.
 4.30 P. M. Time of passage of antrum waves = 29-33 seconds.²
 5.20 P. M. Number of antrum waves per minute = 6.²
 9.30 P. M. Stomach practically empty. Most of the mass in the small intestine. Strong tetany.
 Jan. 29, 9.00 A. M. Some of the mass yet in the ileum; normal movements of ileum; large intestine quiescent; strong tetany.
 C. C. C. = 0.2 M. A.
 2.00 P. M. C. C. C. = 0.1 M. A. Strong tetany, 100 c.c. acid bismuth-starch mass by tube.
 5.30 P. M. Rate of antrum waves, $5\frac{1}{3}$ per minute,² not as deep as normal. Strong tetany.
 6.50 P. M. Stomach practically empty.
 9.30 P. M. Tetany and depression. Killed by ether. Stomach contained two large masses of matted hair and some round worms. Some of the mass in the ileum. There was much unchanged starch in the contents of the ileum and the large intestine.

Cat No. XXIX.

Feb. 14, 1912. Complete thyroidectomy.
 Feb. 15-18. Galvanic hyperexcitability and tetany. Four feedings with standard meat mass.
 Feb. 19, 9.30 A. M. C. C. C. = 0.1 M. A. Strong tetany; temperature, 39.5°; 100 c.c. acid bismuth-starch mass by tube.
 9.40 A. M. Some of the mass throughout duodenum and jejunum.
 2.00 P. M. About one half of mass yet in stomach; antrum waves not as deep as normal; number of waves per minute = $5\frac{1}{2}$ to 6; time of passage of the waves = 36 seconds.
 C. C. C. = 0.1 M. A. Temperature, 38° C. Extreme tetany and epileptic attacks. Stomach and

² This is practically the number and rate of propagation of antrum waves described by CANNON in normal cats. This journal, 1898, i, p. 367.

intestinal movements continue normal during these attacks.

Feb. 19, 4.00 P. M. Condition about as above; stomach practically empty.
6.00 P. M. Small intestine practically empty. Strong tetany and great depression.

Experiment No. X should in all probability be excluded on account of the indigestible masses in the stomach. These solids were undoubtedly responsible for the abnormal delay of the starch mixture in the stomach. In the case of Cat No. XIX, there was one observation similar to the one recorded above on No. XXIX. No. XIX was thyroidec-tomized February 5, developed the usual tetany symptoms and was fed the standard meat mass three times during February 6-9. On February 10 with a galvanic excitability of 0.1 M.A., and with tetany and epileptic attacks and depression 100 c.c. of the acid bismuth-starch mass was given in the usual way by tube. It required five hours for the stomach to pass this mass into the intestine. These two experiments go to show that *in exceptional cases in the last stages of parathyroid tetany when the animals exhibit great depression, there may be a marked diminution in efficiency of the stomach and intestinal motor mechanism.* The artificial chyme may remain in the stomach and in the small intestine more than three times the normal time.

In the case of the remaining thirteen tetany cats the gastro-intestinal motor activities could practically not be distinguished from those of normal cats. The time for the stomach varied from eighty-five to one hundred and forty minutes, with an average of one hundred and ten to one hundred and twenty minutes. The time for complete emptying of the small intestine varied from four and one-half hours to six hours with an average of five and one-half hours. These figures are identical with those obtained on the normal cats. Hence we must conclude that *in the cat even the severe stages of parathyroid tetany usually leave the neuro-muscular mechanism of the stomach and small intestine in normal condition.*

Hyperexcitability of the motor apparatus might result in paralysis from stimulation of inhibitory nervous connections. It seems more probable that it would lead to increased tonus, contracture, inco-ordination, and possibly "peristaltic rush."³ It goes without saying

³ See MELTZER and AUER: this Journal, 1907, xx, pp. 2-9.

that these phenomena were always searched for. All the evidence points to the conclusion that they are not present. Of course, none of the cats were under continuous X-ray observation from the beginning of the tetany to death in convulsions or depression. But the observations were sufficiently frequent and of sufficient duration to note these symptoms if they were in evidence at any stage. Moreover, if in cats parathyroid tetany implied tetany of the digestive tract, the stomach and intestines would be quite as incapable of performing their normal work as is the neuro-muscular mechanism of the skeleton in respect to locomotion.

There does seem to be a slight deviation from the normal in the case of the pylorus mechanism. On the whole the acid as well as the neutral bismuth-starch mixture reached the duodenum after being introduced into the stomach sooner in the tetany cats than in the normal cats. In one tetany cat the mass was observed to pass through the pylorus at once owing to the pressure in the stomach tube and *without any movements of the antrum*. This would indicate a diminished tonus of the pylorus or a slightly diminished sensitiveness of the acid reflexes of the pylorus. This condition may be due to diminished tonus of the vagi fibres rather than to a direct depression of the local mechanisms. This phenomenon is, however, not a constant element in parathyroid tetany, for in some cases the mass did not pass the pylorus until after ten or more minutes, a time interval equal to that in normal cats.

4. *Dogs in various stages of parathyroid tetany.*—The experiments on dogs in tetany were not satisfactory because of the persistent vomiting. It would seem that the stomach of tetany dogs is usually hypersensitive, because the mere passing of the stomach tube, or the introduction of 50 to 100 c.c. of water (cold or at body temperature) usually results in vomiting. The tetany dogs will frequently vomit with nothing but mucus, bile, or intestinal worms in the stomach.

Fairly satisfactory observations were obtained on five dogs. Three of these are given in summary.

Dog No. V.—Weight, 4.4 K.

Dec. 1, 1911. 3.00 P.M. Complete thyroidectomy.

Dec. 2-3. Condition good; no tremors; eats.

Dec. 4. Mild tetany.

May 10

- Dec. 5, 8.00 A. M. Strong tetany.
3.00 P. M. Condition same. 135 c.c. acid bismuth-starch mixtures by tube. In less than five minutes some of the mass found in duodenum and greater part of jejunum. During more or less continuous X-ray observation from 3-4 the antrum and the intestinal movements (segmentation and peristalsis) seemed normal in rate and intensity, while the dog was in strong tetany all the time. Fundus of stomach quiescent.
4.00 P. M. Dog vomited 20 c.c. of the mass.
5.00 P. M. Condition of dog the same; mass distributed throughout the small intestine.
8.30 P. M. Strong tetany; about two thirds of mass yet in stomach. Stomach movements feeble; intestinal movements seem normal.
- Dec. 6, 8.00 A. M. Strong tetany and depression. About one third of mass yet in stomach. No movements of the stomach seen. Segmentation and peristalsis present in the small intestine.
3.00 P. M. Tremors and great depression. Dog bled to death. 30 c.c. of mass (starch unchanged) in the stomach. Duodenum distended with the material in *solid state* and stained with bile. The mass in the rest of the small intestine and in the large intestine more fluid; much unchanged starch present. No ulcers in the stomach and the intestinal mucosa.

Dog No. XIII. — Weight, 3.75 K.

Nov. 29, 1911. Complete thyroidectomy.

Nov. 30-Dec. 2. Condition good.

Dec. 3. Tremors, rapid respiration.

Dec. 4-5. Strong tremors alternating with period of quiescence.

Dec. 6, 8.00 A. M. Tremors; phrenics stimulated by action current of heart; some depression.

11.00 A. M. Condition same. 110 c.c. acid bismuth-starch mass by tube. In less than five minutes some of the mass had reached the duodenum and the upper part of the jejunum; segmentation and peristalsis of the small intestine; feeble peristalsis of the antrum.

Dec. 6, 3.00 P. M. Dog vomited 60-70 c.c. of mass. Antrum movements present.

Dec. 7, 8.00 A. M. Found dead. Stomach contains much bile and mucus. Some of the starch mass in the lower ileum; stomach and intestinal mucosa hemorrhagic.

Dog No. XVIII.—Weight, 5 K.

March 3, 1912. Complete thyroidectomy.

March 4-7. Condition fair.

March 8, 8.00 A. M. Tetany and depression.

2.00 P. M. Condition the same. 150 c.c. acid bismuth-starch mass by tube.

10.00 P. M. Stomach empty; intestinal movements seem normal.

March 9, 8.00 A. M. All the mass in the large intestine; tremors; depression.

The other experiments (Dogs Nos. VIII and X) gave a total time of seven hours and five hours for the stomach. While it is admitted that conclusions based on only five experiments are not convincing, these points may be emphasized:

1. There is no evidence of a tetany (increased tonus, contracture, "peristaltic rush") of the digestive tract.
2. The stomach and the intestinal movements may be initiated by the normal stimuli and persist with normal rate, intensity and co-ordination during severe tetany of the body musculature.
3. The deviation of the motor activities of the digestive tract from the normal in tetany is in the direction of depression and paralysis, and not in the direction of hyperexcitability and increased tonus.
4. Parathyroid tetany usually leads to greater disturbance of the digestive tract (vomiting, motor depression, and paralysis) in dogs than in cats.

II. Gastric digestion in cats in parathyroid tetany.—The observations with the meat feeding in the control and the tetany cats are summarized in Table I. The normal cats show a variation from six hours to eleven hours with an average of eight and one-half hours for the sojourn of the meat in the stomach. The tetany cats show a variation of from ten hours to thirty-plus hours with an average of seventeen and three-quarters hours or about twice the length of time

given to gastric digestion in the normal cats. But these summaries do not convey an adequate conception of conditions and the results in

TABLE I.
SUMMARY OF OBSERVATIONS ON THE TIME REQUIRED FOR GASTRIC DIGESTION OF MEAT
IN NORMAL CATS AND IN CATS IN PARATHYROID TETANY.

Normal cats.			Cats in parathyroid tetany.		
No. of animal.	No. of experiments.	Average time in stomach (hours).	No. of animal.	No. of experiments.	Average time in stomach (hours).
XVIII	5	9.00	XIX	4	10.00
XIX	5	7.50	XX	1	24.00
XXII	2	9.25	XXII	2	30.+
XXIII	2	8.50	XXIV	4	24.+
XXIV	1	7.00	XXIX	2	17.0
XXV	1	10.0	XXXVIII	3	14.0
XXVII	2	80.0	XXXIX	4	14.0
XXIX	1	9.0	XL	2	11.0
XXXV	2	9.0	LV	2	10.5
XXXVI	2	6.25	LX	1	24.0
XXXVII	3	6.50
L	3	8.0
LI	4	11.0
LVI	5	7.50
LIII	4	10.0
LV	2	11.0
Average time 8½ hrs.			Average time 18¾ hrs.		

the tetany group. For that reason the following typical series are given in greater detail:

Cat No. XIX. — Weight, 3.6 K.

Feb. 5, C. C. C. = 1.4 M. A.; complete thyroidectomy.

Feb. 6, 8.00 A. M. C. C. C. = 0.5 M. A.; no tremors.

- Feb. 6, 3.00 P. M. C. C. C. = 0.4 M. A.; no tremors; standard meat mass given forcibly.
 5.00 P. M. All mass yet in stomach; strong tremors.
 10.00 P. M. Some of the mass in duodenum; rest in stomach.
 Feb. 7, 6.00 A. M. Stomach practically empty; tremors.
 4.00 P. M. C. C. C. = 0.25 M. A.; all the mass in large intestine.
 Feb. 8, 9.00 A. M. Strong tetany; salivation; standard meat mass forcibly.
 4.00 P. M. Stomach empty; strong tetany. C. C. C. = 0.2 M. A.
 Feb. 9, 9.30 A. M. Strong tetany. C. C. C. = 0.1 M. A.; standard meat mass forcibly.
 5.30 P. M. Stomach empty; intermittent tetany.
 8.00 P. M. All mass in large intestine; strong tetany.

Cat No. XXII. — Weight, 3.5 K.

- Feb. 6, 10.50 A. M. Cat normal; standard meat mass forcibly.
 4.50 P. M. Stomach empty.
 7.50 P. M. Small intestine empty.
 Feb. 7, 9.00 A. M. Cat normal; standard meat mass forcibly.
 3.30 P. M. Stomach empty.
 6.45 P. M. Small intestine empty.
 Feb. 8, 11.00 A. M. Complete thyroidectomy.
 3.00 P. M. C. C. C. = 1.5 M. A.
 Feb. 9, 2.00 P. M. Slight tremors; C. C. C. = 0.3 M. A.; standard meat mass forcibly.
 8.30 P. M. All mass yet in stomach; no movements of antrum.
 Feb. 10, 8.00 A. M. All mass yet in stomach; C. C. C. = 0.15 M. A.; tremors.
 11.00 A. M. Standard meat mass forcibly; strong tetany.
 4.00 P. M. All mass yet in stomach; strong tetany.
 6.30 P. M. Some of the mass in small intestine; movements of antrum.
 Feb. 11, 9.00 A. M. Tetany and depression; C. C. C. = 0.25 M. A. Temperature, 37° C.; about two thirds of mass yet in stomach; movements of antrum.
 8.00 P. M. Tetany and depression. Temperature, 36° C. Cat killed by ether. 30 gm. of meat mass in a compact ball in fundus of stomach; mass acid throughout and covered with mucus on surface. Meat practically unchanged.

Cat No. XXIV. — Weight, 2.5 K.

- Feb. 8, 9.30 A. M. Cat normal; standard meat mass voluntarily.
 4.30 P. M. Stomach empty.

- Feb. 14, 3.00 P. M. Complete thyroidectomy.
Feb. 15, 9.00 A. M. Condition good; eats; C. C. C. = 1.0 M. A.
Feb. 16, 9.15 A. M. Tremors and labored respiration; C. C. C. = 0.1 M. A.
Standard meat mass forcibly.
8.00 P. M. Only a trace in duodenum, rest of mass in stomach.
Feb. 17, 8.00 A. M. C. C. C. = 0.25 M. A. Tetany; about one fifth of mass yet in stomach.
8.00 P. M. Temperature, 37.5° C. Tetany; standard meat mass forcibly.
Feb. 18, 8.00 A. M. Temperature, 38° C. C. C. C. = 0.1 M. A. All of mass yet in stomach; strong tremors.
8.00 P. M. Mild tetany; temperature 38.3° C.; about one half of mass yet in stomach.
Feb. 19, 8.00 A. M. All of mass in large intestine. Tremors; temperature, 37.5° C.
9.00 A. M. Standard acid bismuth-starch mass by tube.
11.30 A. M. Stomach empty.
4.00 P. M. Small intestine empty.
5.00 P. M. Standard meat mass forcibly.
9.00 P. M. All mass yet in stomach; antrum movements.
Feb. 20, 8.00 A. M. Temperature, 37.2° C. C. C. C. = 0.3 M. A. Tremors and depression; about one third of mass yet in stomach.
3.00 P. M. Stomach empty.
4.45 P. M. Standard acid bismuth-starch mass by tube.
4.48 P. M. None of the mass in duodenum.
7.00 P. M. Stomach empty.
Feb. 21, 9.00 A. M. Temperature, 37.5° C. C. C. C. = 0.3 M. A. Great depression; standard meat mass forcibly; drank some water.
4.30 P. M. Only a trace in duodenum; C. C. C. = 0.3 M. A. Killed by ether. 20 gm. (25 gr. fed) of meat mass, unchanged in stomach.

The following points seem to be established by these experiments:

1. The delay of the meat mass in the stomach in tetany may be the same or only slightly greater than in normal cats, but usually it is much greater.
2. In no case was there an increased rapidity of gastric digestion at any stage of parathyroid tetany.
3. The long delay of the meat in the stomach in parathyroid tetany

is due to deficiency of or change in the gastric juice rather than to motor deficiency. Because in the tetany cats exhibiting this abnormal delay the artificial chyme (acid bismuth-starch mixture) leaves the stomach in the course of about two hours, which is the average time for normal cats.

4. The retarded gastric digestion is not due to the general depression or the moribund condition of the animal at the last stages of the tetany, because it may occur at the very onset of the tetany symptoms.

5. Three factors may operate in this retarded gastric digestion: (a) Direct depression of the gastric gland by toxic substances in the blood; (b) absence of appetite secretion; (c) splanchnic inhibition of the gastric secretion.

III. The effect of section of the splanchnic nerves on gastric digestion in cats in parathyroid tetany. — It does not seem improbable that the sympathetic nervous system should be affected by the conditions that lead to hyperexcitability in the central nervous system and the nerves to the skeletal muscles, in which case the tetany may be accompanied by inhibition of gastric secretion through the splanchnic nerves. However, it would seem that if the tetany would include such a general hyperactivity of the sympathetic inhibitory mechanism there should also be evidence of inhibition of the movements of the stomach and intestines. We have already shown that this is not the case except in the last stage of tetany. But the hypothesis was put to the experimental test. All the splanchnic nerves on both sides were cut on eight cats, the left splanchnics on four cats, and the right splanchnics on two cats. In one cat the major splanchnic nerve was sectioned on both sides, the minor splanchnics being left intact. In one case (Cat No. LX) the section of the splanchnics and the thyroidectomy were made simultaneously. In the rest of the experiments the thyroidectomy was made in three to eleven days after the splanchnic operation to insure physiological degeneration of the cut nerves. The results are summarized in Table II.

The two following experiments are given more fully to show an exceptional condition of accelerated gastric digestion in tetany:

Cat No. XXVIII. — Weight, 2.4 K.

Feb. 10. Section of left splanchnic nerves.

Feb. 13, 8.45 A. M. Standard meat mass voluntarily.

4.45 P. M. Stomach empty.

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Feb. 16, 9.00 A. M. Standard meat mass voluntarily.

9.00 P. M. Stomach empty.

TABLE II.

THE INFLUENCE OF COMPLETE AND PARTIAL SECTION OF THE SPLANCHNIC NERVES
ON GASTRIC DIGESTION IN PARATHYROID TETANY IN CATS.¹

No. of animal.	After section of splanchnic nerves.		After complete thyroidectomy.		Remarks.
	No. of observations.	Gastric digestion (hours).	No. of observations.	Gastric digestion (hours).	
28	3	9½	4	6¾	L. spl. cut.
31	2	7	Both spl. cut. ²
32	3	11½	Both spl. cut. ³
33	3	11	1	24-36	Both spl. cut. ⁴
34	3	10¾	1	18-24	Both spl. cut.
42	2	11	2	7¼	R. spl. cut.
44	1	10	2	16	Both spl. cut.
45	1	10	2	25	R. spl. cut.
46	1	10½	4	13¼	L. spl. cut.
52	2	30	L. spl. cut.
53	4	19	Major spl. on both sides cut.
54	4	20¾	L. spl. cut.
57	1	11	1	16	Both spl. cut.
58	1	13	1	24	Both spl. cut.
59	1	18	Both spl. cut.

¹ Average time of gastric digestion after complete section of splanchnics, 10½; in tetany, 19½; after section of splanchnics on one side, 10¼; in tetany, 17½.
² Died in convulsions 1½ hours after the thyroidectomy.
³ Died in tetany 12 hours after the thyroidectomy.
⁴ Died in tetany 15 hours after the thyroidectomy.

Feb. 21, 9.15 A. M. Standard mass voluntarily.

6.15 P. M. Stomach empty.

8.00 P. M. Complete thyroidectomy.

- Feb. 22. Cat in good condition but does not eat.
- Feb. 23, 9.30 A. M. Standard meat mass voluntarily. C. C. C. = 0.2 M. A.; no tremors.
- 1.00 P. M. Some tremors.
- 2.00 P. M. Stomach empty.
- 3.30 P. M. All of the mass in large intestine; tremors.
- 5.00 P. M. Standard meat mass, in part voluntarily, in part forcibly.
- Feb. 24, 1.00 A. M. Stomach empty; tremors.
- 8.00 A. M. All of mass in large intestine; strong tetany.
- 9.30 A. M. Standard meat mass forcibly.
- 5.30 P. M. Stomach empty. C. C. C. = 0.15 M. A.; tremors.
- Feb. 25, 10.00 A. M. C. C. C. = 0.8 M. A. Temperature, 37° C. Great depression; no tetany; standard meat mass forcibly.
- 4.00 P. M. Stomach empty.
- 7.30 P. M. Cat died; stomach and small intestine empty.

Cat No. XLII.

- March 4. Section of splanchnic nerves on right side.
- March 7, 9.45 A. M. Standard meat mass forcibly.
- 10.00 P. M. Stomach practically empty.
- March 10, 1.00 P. M. Standard meat mass forcibly.
- 11.00 P. M. Stomach empty.
- March 11, 5.00 P. M. C. C. C. = 1.5 M. A. Complete thyroidectomy.
- March 12, 8.00 A. M. Temperature, 37.5° C. Depression; no tremors; standard meat mass forcibly.
- 6.00 P. M. Stomach empty; depression; diarrhea.
- March 13, 2.00 P. M. Temperature, 36.5° C. Depression; no tremors. C. C. C. = 0.6 M. A. Standard meat mass forcibly.
- 6.50 P. M. Stomach empty. Killed with ether. The mass in jejunum and ileum seems well digested.

The following points in this group of experiments may be emphasized:

1. On comparison of Table I and Table II, it would seem that partial and complete section of the splanchnic nerves slightly retards gastric digestion as compared to that in normal cats. This is contrary to Cannon's observations.⁴ It is probable that the greater delay in our

⁴ CANNON: this Journal, 1906, xvii, p. 429.

operated cats is due to the fact that most of the feeding experiments were made before complete healing of the abdominal wound, and while abdominal adhesions were in process of formation. These conditions would necessarily produce some discomfort and depression.

2. With the exception of two experiments (Nos. XXVIII and XLII) the retardation of gastric secretion and digestion in parathyroid tetany is just as marked in cats with complete or partial section of the splanchnic nerves as in cats with these nerves intact. It would thus seem clear that inhibition of the gastric glands through the splanchnic nerves is not a factor in this delay. It must be noted, however, that inasmuch as complete thyroidectomy after previous complete section of the splanchnics results in greater depression and quicker *exitus* than does the same operation in normal cats, the influence of this general depression and the rapidly developing moribund condition as factors in the retarded gastric digestion may be greater in the former group than in the latter. It was proved again that the delay was not due to motor insufficiency by giving the artificial chyme (acid bismuth-starch mass) by tube (Experiments Nos. LIII and LIV). This mass was passed out of the stomach in two hours, or about normal time, while the meat mass was retained by the stomach more than twice the normal time.

3. There are two instances in this group in which the gastric digestion in tetany is more rapid than the normal. In each case the stomach time was four and one-half hours, which is one and one-half hours less than the most rapid gastric digestion observed in any normal cat in my series. In one of these cases the meat was taken voluntarily, and presumably with appetite secretion; in the other it was given forcibly. One of my normal cats weighing 2.5 K. showed consistently a six-hour period for the stomach on 25 gm. of the meat. This cat was nursing three two-week-old kittens. She had an unusual appetite, and took 100 to 150 gm. of meat each day in addition to the meat-bismuth mixture. Cannon's extensive experiments on cats, with practically the same quantities of meat (cooked), show the stomach time to be at least six to seven hours.⁵ Hence there can be no question but that in these two cases *the gastric digestion was accelerated by the tetany condition*. We assume, provisionally, that this was due to increased secretion of gastric juice. On this point, then, our results so far indi-

⁵ CANNON: this Journal, 1905, xii, p. 387.

cate that the tetany is usually accompanied by retardation of gastric secretion, but in exceptional cases it may be normal and even greater than normal.

Analogous conditions obtain for the salivary glands in parathyroid tetany, with this difference that the hypersecretion is the usual phenomenon. Both in cats and dogs the tetany is usually accompanied by salivation. But in some cases there is no distinct salivation, although the mouth and lips appear to have the normal moisture. So far I have observed in three dogs and a number of cats in all stages of tetany the salivary secretion completely stopped so that the mouth and lips became dry and parched, showing complete failure of the salivary reflexes.

4. In cats complete section of the splanchnic nerves seems to diminish the resistance to subsequent thyroid-parathyroidectomy. The tetany appears sooner, but is less intense or violent, the depression symptoms being more pronounced, and the animal dies sooner in depression or epilepsy. The eight cats survived the thyroid parathyroid operation on the average thirty-four hours, the extremes being one and one-half hours and sixty-five hours respectively. The cat that survived the longest had the splanchnic sectioned and parathyroid removed at the same time. The cat that died within one and one-half hours had recovered from the ether and was walking about in apparently normal condition. The thyroid operation on cats requires only ten minutes' light anaesthesia. In all my experience no normal cat has died from the anaesthesia or operation trauma. Still, it is difficult to conceive how the tetany condition could have developed to a fatal culmination in ninety minutes. These results on cats with the splanchnics sectioned are similar to those obtained by Vincent and Jolly⁶ on normal foxes.

In the way of explanation of the fact two working hypotheses suggest themselves, viz. (a) the low systemic blood pressure may lead to deficient circulation in the central nervous system, and in consequence to an accumulation of metabolites and a lowered resistance; (b) the lesion of the splanchnic nerves may lead to changes in the activity of some of the visceral organs analogous to or in the same direction as in the tetany. These hypotheses have not yet been put to the test of experiments.

⁶ VINCENT and JOLLY: *Journal of physiology*, 1905, xxxii, p. 65.

IV. On the importance of the appetite secretion of gastric juice in the gastric digestion in cats. — Inasmuch as practically all the meat feedings in the tetany cats were forced feedings, the animals having no appetite, or at least not being willing to eat, there remains to be considered the possibility that the great delay in the gastric digestion of meat in tetany is due to the absence of appetite secretion. We have seen it is not due to splanchnic inhibition.

So far as I know, the importance of appetite secretion in the cat has not yet been made the subject of direct investigation. But one would not expect a great difference in this regard between the cat and the dog, and according to the extensive work of Pawlow and his school, lack of appetite secretion in the dog would lead to a considerable retardation of the gastric digestion. The same is true for man.⁷ Cannon's observations on gastric peristalsis after section of both vagi are of interest in this connection.⁸ Appetite secretion of gastric juice is, of course, impossible after section of both vagi. Yet Cannon found that within a few days after this operation in cats the rate of discharge of the food stuffs from the stomach was practically the same as in normal cats. The same was true after section of both the vagi and the splanchnics. Immediately after double vagotomy the food remains in the stomach longer than normally, but the recovery is too quick to be accounted for by regeneration of the nerves. Cannon discusses his results solely from the point of view of gastric peristalsis and pylorus control. But it is obvious that the rate of emptying of the stomach after feeding solid food depends as much on the rate of gastric digestion as on the rate and strength of the gastric movements.

In the case of our control cats some would eat the standard meat mass with great appetite, others had to be fed forcibly. The same cats might eat voluntarily one day and a day or two later have to be fed forcibly. Most of the cats exhibited this variability despite the fact that in nearly every case food was withheld from the animal for twenty-four hours or more before the experiments. A normal cat may thus go without food for thirty-six hours, and yet refuse to eat raw lean meat to which are added a little bismuth subnitrate and a little peptone. If such a cat is hungry, it is difficult to see why it does not

⁷ HAUDEK and SIGLER: *Archiv für die gesammte Physiologie*, 1910, cxxxiii, p. 145.

⁸ CANNON: *this Journal*, 1906, xvii, p. 429.

eat unless the food offered is not to its taste. And if the latter is the case, giving this food forcibly ought not to lead to appetite gastric juice. When one compares the present data on the control cats, the gastric digestion in the cats fed forcibly is just as rapid as in those eating voluntarily, as the following figures show:

	Number of feedings.	No. of cats.	Average time in stomach.
Forcible feedings . . .	18	12	8.7 hours
Voluntary feedings . . .	22	11	8.3 hours

The point might be made that the importance of the factor of appetite gastric secretion would be minimized by the fact that peptones were added to the meat. A few experiments were made to test this objection using dog and cat muscle instead of ox muscle and dispensing with the peptone. The cats so far tried will not eat cat or dog meat unless starved for two or more days. The results so far obtained seem to show that cat and dog meat given forcibly passes out of the stomach within eight to nine hours, or within the same period as ox meat eaten voluntarily. It would thus seem that "appetite gastric secretion" does not play as important a rôle in the cat as Pawlow found in the case of the dog, or it is possible that the importance of the appetite gastric secretion in the dog has been exaggerated.

In order to determine whether the increased delay of the food in the stomach after section of the vagi is solely a matter of motor deficiency or in part due to lack of appetite secretion, both vagi were sectioned in four cats and subsequently the standard acid bismuth-starch mass was given by stomach tube. One of these experiments may be given in summary.

Cat No. XLVII. — March 5, section of left vagus; March 8, section of right vagus.

March 10, 12.30 P. M.	Standard acid bismuth-starch mass by tube.
8.30 P. M.	Stomach empty.
March 12, 4.00 P. M.	Standard acid bismuth-starch mass by tube.
7.30 P. M.	Stomach empty.
March 14, 8.00 A. M.	Standard acid bismuth-starch mass by tube.
10.45 A. M.	Stomach empty.

These results seem to show that most of the increased delay of the food in the stomach after vagi section is due to motor rather than to secretory insufficiency. The importance of the gastric appetite secretion in the cat must, of course, be determined by direct experiments, but the data so far at hand suffice to prove that the great retardation of gastric digestion in tetany is not due solely to the absence of appetite secretion. And since the delay is not caused by splanchnic inhibition, we are forced to the conclusion that it is due to diminished or altered secretion owing to direct depression of the gastric glands or the local secretory reflex mechanisms.

V. Gastric digestion in parathyroid tetany in dogs. 1. *Rate of gastric digestion of meat in normal dogs.* — Eighteen feeding tests were made on five normal dogs using the well-ground lean meat (plus the bismuth and peptone) in quantities of 10 gm. per kilo body weight. In all of these tests the dogs ate the food with great appetite, and presumably, therefore, with accompanying appetite secretion. *The delay of the meat in the stomach varied from eight hours to fourteen hours with an average of ten and one-half hours.* The following is a typical series:

Dog No. XXI. — Weight 4 K. 40 gm. of the standard meat mixture eaten voluntarily.

April 23, 10.00 A. M.	Fed	} 12 hours.
10.00 P. M.	Stomach empty	
April 24, 10.30 A. M.	Fed	} 10 hours.
8.30 P. M.	Stomach empty	
April 25, 9.00 A. M.	Fed	} 8 hours
5.00 P. M.	Stomach empty	
April 26, 8.30 A. M.	Fed	} 13 hours.
9.30 P. M.	Stomach empty	
April 27, 9.00 A. M.	Fed	} 10 hours.
7.00 P. M.	Stomach empty	

Average time for the gastric digestion, ten hours and forty-one minutes.

2. *Rate of gastric digestion in dogs in tetany. The condition of the appetite.* — As a rule dogs in parathyroid tetany do not eat voluntarily. There is no exception to this rule in the case of severe tetany. Such dogs may lap water voluntarily, but they will not or cannot eat, and it is practically impossible to feed them forcibly because of vomit-

ing. Many dogs will eat voluntarily in the interval between the tetany periods. These intervals vary in length from three hours to thirty-six hours. I have so far observed only six dogs eating voluntarily when in mild tetany and tremors. In my experience dogs in marked depression following the tetany attacks do not eat. Because of these facts forcible feeding had to be employed in most of our tests. This evidently implies absence of appetite gastric secretion.

All stages of parathyroid tetany retard the gastric digestion in dogs. — In the animals that retained the meat in the stomach long enough for recording the digestion, the time varied from eighteen hours to more than forty-eight hours. The latter is practically a suppression of gastric digestion. The retardation of digestion is in evidence but not extreme, even when the meat is eaten voluntarily in mild tetany or in periods of quiescence between the tetany attacks. Two dogs retained the meat for two days and five dogs for more than one day before vomiting. These are exceptions, however. In fact, most of our observations consisted in recording that all or nearly all of the meat was retained in the stomach between the interval of feeding and the interval of vomiting. If this interval is from five to ten hours, it is obvious that the gastric digestion is retarded, although we cannot give the amount of retardation in hours. I have observed the same intense peristalsis of the antrum prior to vomiting in tetany dogs that Cannon described in normal dogs. Two experiments were performed on dogs after section of the splanchnic nerves and complete thyroidectomy.

Dog No. XXV. — Weight, 5 K.

- April 16, 11.00 A. M. Section of the splanchnic nerves on both sides.
 April 22, 11.00 A. M. Complete thyroidectomy.
 April 23, 10.00 A. M. No tremors. 50 gm. meat voluntarily.
 4.00 P. M. Tetany and rapid respiration; about two thirds of meat yet in stomach.
 7.00 P. M. Tremors; about one half of meat yet in stomach.
 11.00 P. M. About one third of meat yet in stomach.
 April 24, 10.00 A. M. Tremors and depression; all meat mass in large intestine; 50 gm. meat voluntarily.
 4.00 P. M. Strong tetany; all meat yet in stomach.
 10.00 P. M. Strong tetany; two thirds of meat yet in stomach.
 April 25, 8.00 A. M. Stomach practically empty; tremors and depression.

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- April 25, 9.00 A. M. 50 gm. meat voluntarily.
5.00 P. M. Two thirds of meat yet in stomach.
April 26, 8.00 A. M. One fourth of meat yet in stomach; no antrum movements; mild tetany.
3.00 P. M. Condition same.
11.00 P. M. About one tenth of meat yet in stomach.
April 27, 9.00 A. M. Stomach empty; mild tetany.
10.00 A. M. 50 gm. meat forcibly.
11.00 A. M. Vomited all the meat plus bile and mucus.
April 28, 9.00 A. M. Depression, some tremors; temperature, 35° C. Killed by ether. Bile and mucus in stomach. Intestinal mucosa hemorrhagic. No lesions in stomach mucosa.

Dog No. XXVIII.—Weight 4.9 K.

- April 15. Section of the splanchnic nerves on both sides.
April 22. Complete thyroidectomy.
April 23, 10.00 A. M. Condition good; 50 gm. meat voluntarily.
8.00 P. M. Stomach empty.
April 24, 9.30 A. M. Violent tetany; rapid respiration; salivation.
11.30 A. M. Dog recovered.
2.00 P. M. No tetany; 50 gm. meat voluntarily.
4.00 P. M. Strong tetany.
10.00 P. M. Mild tetany; nearly all the meat yet in stomach.
April 25, 8.00 A. M. Stomach nearly empty; no tremors.
9.00 A. M. 50 gm. meat voluntarily.
5.00 P. M. Strong tetany; three fourths of meat in stomach.
10.00 P. M. About one half of meat yet in stomach.
April 26, 8.00 A. M. Stomach and upper part of duodenum empty; rest of the mass in jejunum, ileum, and large intestine; no tremors; depression.
4.00 P. M. 50 gm. meat forcibly.
11.00 P. M. Tetany; all the meat yet in stomach.
April 27, 8.00 A. M. About one half of meat yet in stomach. Mild tremors.
4.00 P. M. One fourth of meat yet in stomach.
10.00 P. M. Stomach nearly empty.
April 28, 11.00 A. M. Depression; no tetany; 50 gm. meat forcibly.
11.00 P. M. Nearly all the meat yet in stomach.
April 29, 10.00 A. M. Vomited about 35 gm. of the meat, largely unchanged. Depression; no tetany; does not eat. Experiment discontinued.

The two dogs with previous section of the splanchnic nerves exhibited the typical retardation of gastric digestion. *It is therefore not a phenomenon of splanchnic inhibition.* The tetany and depression symptoms were not augmented, as was the case in the cats with previous section of the splanchnics. But the two dogs showed much greater appetite and less vomiting than is usually the case in tetany dogs with the splanchnics intact. It is not worth the while to comment on this, in view of the fact that only two experiments are so far at hand. It may be of interest to note, however, that Dog XXV ate the meat *voluntarily* on February 23, 24, and 25, and Dog XXVIII ate voluntarily on February 24 and 25, notwithstanding which there was great delay in the gastric digestion in each case. The lack of appetite gastric secretion is therefore not an important factor in this delay.

VI. The condition of other sympathetic nervous mechanisms in parathyroid tetany. 1. *The cervical sympathetic nerves.*—(a) In the extreme parathyroid tetany or epileptic attacks there is always the dilation of the pupils typical of other forms of epilepsy, but in moderate or strong tetany there is no evidence in the pupils of an increased tonus of the cervical sympathetic fibres. To be sure, I have had dogs in moderate tetany and depression showing somewhat dilated pupils, but as this may be the case even after section of the cervical sympathetics, it must be due to a lowered excitability of the retina or to depression of the reflex centres. It is well known that parathyroid tetany is sometimes accompanied by temporary blindness.

(b) The salivation of parathyroid tetany is not due to over-stimulation of the cervical sympathetic nerves, at least in the cat and the dog, for section of these nerves does not influence the course of the salivation. Inasmuch as the salivation is not a constant factor in the tetany, the absence of salivation after section of the cervical sympathetic nerves is of no significance. I have had such cases. But three cats and two dogs showed the usual salivation, although the nerves were sectioned at the time of the parathyroidectomy. No further attempt was made to determine the mechanisms involved in the salivation of tetany. The cranial secretory fibres are probably involved rather than a direct chemical action on the salivary gland cells.

2. *The pilo-motor nerves.*—At no stage of parathyroid tetany have I ever observed pilo-motor action in dogs or cats. I have seen it in

the last stage of extreme tetanic attacks that ended in death, but in such cases it is in all probability due to asphyxia.

3. *Sweat secretion.* — Distinct secretion of sweat in tetany was observed in one cat. This cat did not show pyrexia, so that the sweating could not have been due to the normal reflexes. Sweating must therefore be regarded as a very exceptional phenomenon in parathyroid tetany in cats. Sweating in tetany cannot be considered an evidence of hyperexcitability of sympathetic nerves, unless the body temperature is normal or sub-normal. In case the body temperature is elevated the normal heat regulating mechanisms may be brought into play.

4. *The bladder.* — There is no incontinence of the urine in parathyroid tetany, except in very strong attacks, when there may be occasional spurts or dribbling from the urethra. The great pressure on the bladder from the abdominal muscles is probably responsible for this rather than an increased excitability of the neuro-muscular mechanisms of the bladder.

5. *The rectum and the internal sphincter of the anus.* — When the tetany animals have diarrhea, there are the usual frequent movements of the bowels. In these cases there may be greatly diminished tonus both of the internal and the external sphincters. When there is no diarrhea, the defecations are always less frequent than in normal animals. This appears to be true even when no bismuth is added to the food. Hence parathyroid tetany does not seem to induce hyperexcitability of the neuro-muscular apparatuses of defecation.

6. *The condition of the pregnant uterus in tetany.* — In the course of another line of investigation together with Dr. A. Werelius complete thyroidectomy was performed in ten dogs and eight cats nearing full-term pregnancy. With the exception of one dog and one cat all these animals developed tetany varying in degree from mild tremors to extreme epilepsy. After twenty-four hours in tetany the labor started in one of the dogs and three full-sized pups were born in the course of twelve hours. The dog died thirty-six hours later in depression. Five pups (fully developed) were found in the uterus. The head of one of the pups was engaged, but evidently there had not been enough of force in the uterine contractions to expel it. In this case the condition of the external genitalia and the size of the pups showed the mother to be at term, and we can therefore not infer that the tetany induced the labor. The evidence points rather to the reverse conclusion, that the tetany

condition rendered normal labor impossible by depression of the neuromuscular mechanisms of the uterus. All of the cats and the rest of the dogs died without giving birth to their young, although some of them certainly were at term, and all the fetuses were alive at the time of the death of the mothers. In no case were uterine contractions detected by palpation. The membranes were not ruptured.

It is well known that as a rule eclampsia convulsions in the human do not induce labor. The same conditions appear to obtain for parathyroid tetany in dogs and cats. Instead of producing hyperexcitability and contraction of the pregnant uterus, the parathyroid tetany seems to lead to depression of the uterus.

7. *Condition of the emotions.* — Evidence of pain, lack of appetite, and depression are most constant symptoms. But moderately strong tetany may be present — particularly in dogs — without any obvious sympathetic activity efferent or afferent. Dogs may respond to caresses, fight a cat, or eat with evident relish. In one case I observed a male dog in parathyroid tremors of sufficient violence to render locomotion very uncertain attempt to cover a bitch in rut.

8. *The concentration of adrenalin in the blood* could probably be used as a criterion of hyperactivity of the sympathetic nervous system in tetany.⁹ But in view of the fact that the physiological tests for adrenalin are not strictly specific, and the probability that the tetany involves important changes in the blood besides in the possible adrenalin content, the results would not be conclusive. The increase in undetermined urinary nitrogen in tetany is at least suggestive of such blood changes. There is also a very great increase in the urinary diastase. It seems, therefore, that the changes in the blood in tetany are too complicated or too little known to yield anything of value by the adrenalin method.

DISCUSSION OF RESULTS.

The methods employed in this work aimed at studying the activities of the visceral organs so far as possible under conditions of normal stimulation. The results show in general that *in tetany in cats and dogs the deviations from normal activity are in the direction of depression, not in the direction of hyperexcitability or hyperactivity.* The later con-

⁹ CANNON and HOSKINS: this Journal, 1911, xxix, p. 274.

dition of the viscera in tetany is a rare exception. This may or may not be true for other genera.

In the case of other forms of tetany in the human there appears to be tetany of the visceral as well as of the skeletal musculature, and also hyperactivity of the digestive gland. Ibrahim¹⁰ records tetany of the sphincters, the smooth muscle, and the heart in infantile tetany. The very extensive study of clinical tetany with reference to the visceral organs by Falta and Kahn¹¹ appeared during the progress of the present investigation. These investigators describe spastic constriction of the stomach, spasms of the ciliary muscle and of the blood vessels, angiospastic edema, hypersecretion of sweat, saliva, tears, gastric and intestinal juice, as usual symptoms of tetany in man. They record a hypersensitiveness of the vascular system to adrenalin, and of the digestive tract to pilocarpine as further evidence that the tetany involves hyperactivity of the sympathetic and the autonomic nervous mechanisms. The picture of *tetania gastrica* described by Falta and Kahn in clinical tetany does not resemble the visceral conditions in experimental parathyroid tetany in cats and dogs. Of course, it is still an open question whether all forms of clinical tetany represent parathyroid insufficiency. There are certainly many cases of infantile tetany without any demonstrable lesions in the parathyroids.¹² And there may or may not be abnormal dilation of the stomach in infantile tetany.¹³

I have never seen any indication of angiospastic edema in my tetany animals. But it must be admitted that this symptom would be more difficult to detect in animals with fur than in man. Increased sweating was observed in only one cat and pilo-motor activity at no time. The secretory and motor fibres to the stomach in the vagi lie just as close to the heart as the phrenic nerves; yet in parathyroid tetany the latter are stimulated by the action current of the heart, the former not.

Angiospasmms ought to diminish the secretion of urine by diminishing the blood flow through the kidneys. There seems to be no diminished

¹⁰ IBRAHIM: Deutsche Zeitschrift für Nervenheilkunde, 1911, xli, p. 436.

¹¹ FALTA and KAHN: Zeitschrift für klinische Medizin, 1911, lxxiv, p. 108.

¹² BLISS: Zeitschrift für Kinderheilkunde, 1911, ii, p. 538; JÖRGENSEN: Monatsschrift für Kinderheilkunde, 1911, x, p. 184.

¹³ THORSPEEKEN: *Ibid.*, p. 420.

kidney activity in tetany if the animals take water voluntarily or if the normal amount of water is given them by stomach tube. The early observations of Smith¹⁴ on parathyroid tetany in cats are of interest in this connection. Smith found that the tetany in cats is accompanied by subnormal temperature. In only one case did he find a slightly elevated temperature in strong tetany. He showed further that this lowered temperature is due to increased heat loss rather than to diminished heat production. The heat loss is not through evaporation of sweat; it is probably due to depression or paralysis of the vaso-constrictor mechanism of the surface blood vessels, a condition of depression rather than of hyperexcitability of the sympathetic system.

I have confirmed Smith's results as to the subnormal temperature in tetany cats. In no case did I find an increased temperature, even in the most violent tetany. Tetany and galvanic hyperexcitability may exist with a body temperature as low as 34°-35° C. On this point there is a striking difference in the tetany in dogs and cats. Strong tetany in dogs is always accompanied by increased temperature, while mild tremors and depression may coexist with subnormal temperature.

There seems to be nothing in the present results that might give a clue to the cause of the usual anorexia in parathyroid tetany. Lack of hunger may come about (1) by quantitative or qualitative change in the afferent impulses in the sensation; (2) interference by abnormal or abnormally strong impulses from other afferent paths, or (3) by change in the part of the encephalon concerned with the processes of hunger sensation. In most of the tetany dogs the lack of appetite may be due to or associated with the evident abdominal distress and possibly the nausea associated with the vomiting. But the anorexia may be present in dogs which do not vomit or show signs of abdominal pain. Vomiting is a rare exception in tetany cats, but the anorexia is invariably present. The recent work of Cannon and Washburn¹⁵ seems to give strong support to the theory that gastric contraction initiates the hunger sensation. This theory would account for the anorexia in the later stages of tetany when there is great motor deficiency of the digestive tract. But the lack of hunger is just as marked (at least in cats) when the gastric motor mechanisms remain, to all appearance,

¹⁴ SMITH: *Journal of physiology*, 1894, xvi, p. 378.

¹⁵ CANNON and WASHBURN: *this Journal*, 1912, xxix, p. 441.

normal. In practically all of the cats and dogs there is evidence of diminished or altered gastric secretion. Yet in exceptional cases dogs with their gastric glands in such condition will eat. I agree with Cannon and Washburn that the fact of an animal eating is not conclusive evidence that the animal is hungry. Man may eat (1) because of hunger, (2) because of taste for special foods, (3) from habit, (4) from a sense of duty ("eating to keep alive"). We have no means of knowing whether or not all of these factors must be considered in experimental animals. But while it is true for man and may be true for dogs and cats that one may eat without being hungry, we think most physiologists will agree that a hungry animal will eat if food is available; that failure to eat is therefore a criterion of the absence of hunger; and that only a very hungry animal will eat impalatable or disgusting food. It would thus seem that in the eating of impalatable or disgusting food we have a reliable objective criterion for the presence of hunger of marked degree in experimental animals. This test has failed to show hunger in all the tetany animals so far examined, but the experiments are being continued and extended to animals in which the afferent nervous paths to the viscera are eliminated in the hope of determining more precisely the peripheral and the central factors in hunger.

It is not clear to what extent the changes in the digestive glands indicated by our results are primary or secondary in the tetany. This point is being investigated on dogs with Pawlow's accessory stomachs by Dr. H. E. French. But the retardation of digestion may explain the aggravation of the tetany by protein feeding. The feeding must lead toward increased bacterial action, and absorption of bacterial toxins as well as toxic splitting products of the proteins.

A possible criticism of the method of investigation followed in most of the experiments occurred to me at the end of the work. There may be change in the intestinal absorption in tetany so that the bismuth salt may be taken up in toxic quantities. This might be an important factor in tetany dogs, because of the usual hemorrhagic condition and ulcers in the stomach and intestines. But in no case did I find ulcers or distinct hemorrhagic areas in cats. However, the above criticism can only be met by a study of intestinal absorption in tetany animals.

The marked difference in certain of the tetany symptoms in dogs and

cats is rather surprising in two so closely related groups. The difference may be represented in the following manner:

Symptoms.	In tetany dogs.	In tetany cats.
1. Body temperature. . .	Usually pyrexia	Usually hypothermia.
2. Gastero-enteritis . .	Usually present	Usually absent.
3. Vomiting	Usually constant	Practically absent.
4. Periodicity in the motor symptoms . . .	Usually distinct	Practically absent.

Symptoms subject to such variations must be regarded as secondary effects of parathyroid deficiency depending on some peculiarity of the species, or on the special physiological condition of the individual. Much more work must be done on all available species before the secondary and non-essential symptoms are eliminated from the parathyroid tetany syndrome.

SUMMARY AND CONCLUSIONS.

1. There are no spasms, contractures or other evidence of hyperexcitability or tetany of the neuro-muscular mechanisms of the digestive tract in parathyroid tetany in cats and dogs. Even in very severe tetany the movements of the stomach and the intestines may be normal. The deviation from normal is in the direction of depression or paralysis.

2. The indirect methods used in this work indicate that the gastric and pancreatic digestion in tetany may be normal; but it is usually retarded. The retardation may amount to practical failure of digestion. In very exceptional instances there may be acceleration of the gastric digestion (cats). It is shown that the retarded digestion is not due to the absence of appetite secretion or to splanchnic inhibition. It is probably due either to direct action of substances in the blood on the digestive glands (secondary effects) or to altered activity as a direct effect of the absence of the parathyroid secretion.

3. In the case of other sympathetic and autonomic mechanisms (cervical sympathetic, pilo-motors, sweat nerves, the uterus, the bladder, the sphincters) the deviation from normal activity in parathyroid tetany in cats and dogs seems also to be in the direction of depression.

GLYCOLYSIS AFTER PANCREATECTOMY AND WITH THE ADDITION OF ANTISEPTICS.

BY HUGH MCGUIGAN AND C. L. VON HESS.

[From the Pharmacological Laboratory of Northwestern University Medical School.]

A MIXTURE of normal muscle extract with pancreatic extract has no greater glycolytic power than these extracts taken separately.¹ However, there may be sufficient internal pancreatic secretion in normal muscle so that its extract exerts a maximal action on glucose; for this reason the present investigation has been an attempt to develop a condition of pancreatic hunger in muscle and to determine, then, the effect of adding pancreatic extract.² Accordingly the glycolytic action of muscle extracts of both normal and pancreatectomized animals has been tested, and it may be stated in the beginning that, by the methods used, no difference has been found in the action of the muscle extracts either alone or when mixed with pancreatic extract.

METHODS.

The pancreas was removed completely from a number of dogs under aseptic precautions. The animals rallied quickly; sugar was always found in the urine within twenty-four hours after the operation and persisted until the dogs were killed. Post mortem examination showed that in all cases the removal of the pancreas was complete.

Preparation of the extracts.—The dogs were bled to death under ether anaesthesia. The muscles of normal dogs were removed, ground, triturated in a mortar to a fine pulp and macerated in an ice chest from four to twelve hours, when the juice was obtained by a hand press. In the experiments after pancreatectomy the pressure juice was obtained

¹ MCGUIGAN: this Journal, 1908, xxi, p. 351.

² Our attention was drawn to this point by Dr. R. T. WOODYATT. OPPENHEIMER (Die Fermente, 1910, p. 490) also suggests such a possibility.

by the aid of a Buchner press at 300 atmospheres. Either NaHCO_3 , MgCO_3 , or phosphate solution (1 part monosodium and 9 parts disodium in 100 parts of H_2O) was added to preserve neutrality and the mixture made to a definite volume. For the pancreas extract a fresh organ of the same species was ground, macerated in water, and filtered through cheese cloth. In one or two instances the residue was repeatedly extracted with alcohol and the combined extracts evaporated on a water bath to dryness, taken up with water and filtered and made to a known volume as recommended by Hall.³

Conduct of experiments. — Glucose was added to the muscle extract, definite volumes of the latter measured into Erlenmeyer flasks, and varying amounts of the pancreatic extracts added. An antiseptic, as indicated in each case, was added and the mixtures incubated at about 40°C . (37° – 42°). At the beginning and the end of experiments the sugar was determined by the Pavy method. A control was used in each case. The proteins were removed with acetic acid and sodium sulphate, the excess of acid being neutralized with ammonia, before titration.

The figures in Tables I and II indicate very plainly that, even under optimal conditions for glycolysis, this method fails to show either the presence or the influence of an internal pancreatic secretion. This fact does not disprove the theory advanced by Cohnheim,⁴ but it does argue against the validity of the results and the method used to support them. The glycosuria following the removal of the pancreas and the work of Drennan,⁵ Hédon,⁶ Scott,⁷ Zuelzer,⁸ and others still supports the theory that an internal secretion of the pancreas aids the consumption of the sugar by the muscles.

The glycolysis occurring in the blood and in solutions of dextrose to which yeast has been added is generally considered to be identical with the glycolysis which occurs normally in the muscles. If this be

³ HALL: this Journal, 1907, xviii, p. 280.

⁴ COHNHEIM: Zeitschrift für physiologische Chemie, 1903, xxxix, p. 336; 1904, xlii, p. 401; 1905, xliii, p. 547; 1906, xlvii, p. 253.

⁵ DRENNAN: this Journal, 1911, xxviii, p. 306.

⁶ HÉDON: Comptes rendus de l'Académie des Sciences, 1911, lxxi, No. 25, p. 124.

⁷ SCOTT: this Journal, 1912, xxix, p. 306.

⁸ ZUELZER, DOHRN, and MARXER: Berliner klinische Wochenschrift, 1908, p. 1380.

true, then the action of chloroform and toluol in preventing this glycolysis would explain the failure to obtain any evidence of glycolysis with muscle extracts, even though glycolysis occurs there normally. The following experiments (Table III) will illustrate:

Flask B gave no indication of bacterial growth during the first thirty hours, but after seventy-two hours smelled foul. This probably accounts for the more rapid glycolysis during the last six hours of the experiment.

It is to be noted that both normal and added sugar is used in glycolysis. If the same mechanism operates in the tissues, then free sugar can be utilized directly. This agrees with previous work.⁹

Toluol or chloroform, in the concentrations used here, stops glycolysis. Formaldehyde, even in relatively strong concentrations, inhibits but does not prevent the action. The added sugar is glycolyzed as well as the normal sugar of the blood. If the action be due entirely to enzymes, we should not expect such a great inhibition as occurs here, since enzymes are much less affected by antiseptics.

The glycolysis by yeast is similarly influenced. One per cent toluol or chloroform stops the action of yeast, and the action of the yeast pressure juice is also greatly inhibited. Formaldehyde does not completely stop the action of yeast until added to a concentration of one half per cent.

Chloroform, toluol, or both have been recommended and used to check bacterial growth in most work where the glycolytic power of the tissues is tested, but since the glycolytic action of blood and of yeast is considered similar to that occurring normally in the muscles and these processes are stopped entirely by the concentrations of antiseptics used, and since weaker concentrations will allow the growth of bacteria, one must conclude that *the antiseptic method is absolutely inadequate to furnish evidence in favor of the theory that the muscles require the internal secretion of the pancreas in the utilization of sugar.*

Regarding the differences between the action of antiseptics on the cell and on enzymes, the evidence indicates clearly that in many cases there is a wide difference. Cells may be killed without seriously injuring the enzyme. Since the continued enzymic action depends upon its constant formation by the cell and no change in enzymic action can be found when the metabolism of sugar is seriously disturbed,

⁹ McGUIAN: this Journal, 1908, xxi, p. 334.

EXPERIMENT 5. — YOUNG DOG.												
2	50	Hand press	Phosphate solution	68	2	Hall's method	0.4	43 hr.	Toluol	0.510	0.015
3	50			68	3		0.6			0.503	0.017
4	50			68	4		0.8			0.510	0.015
5	50			68	6		1.2			0.510	0.015
6	50			68	8		1.6			0.510	0.015
7	50	Hand press	MgCO ₃	68	10	Hall's method	2.0	21 hr.	Toluol	0.512	0.013
8	50			68	0.512	0.013
0	50	Hand press	MgCO ₃	35	Hall's method	21 hr.	Toluol
1	50			35	0.1		0.01			0.055
2	50			35	0.2		0.02			0.045
3	50			35	0.4		0.04			0.075
4	50			35	0.6		0.06			Lost
5	50			35	1.0		0.10			0.029
6	50			35	2.0		0.20			0.029
7	50			35	4.0		0.40			0.052
8	50			35	6.0		0.60			0.031
9	50	35	10.0	1.00	0.052					

TABLE II.
PANCREATIC ACTIVATION OF DEPANCREATIZED MUSCLE.

Flask No.	Muscle extract.	Obtained by	Neutralized by	Muscle represented.	Pancreatic ext.	Preparation.	Pancreas represented.	Incubation at 40°.	Antiseptic used.	Sugar content at beginning.	Sugar content at end.	Glycolysis.	Remarks.
0	50			22						0.455			
1	50			22	1		0.14			0.442	0.013	
2	50			22	2		0.28			0.419	0.036	
3	50			22	3		0.43			0.428	0.027	
4	50			22	4		0.57			0.437	0.018	
5	50	Press at 300 at.	MgCO ₃	22	5	Water extract	0.71	48 hr.	Toluol	0.464	-0.009	Seven days after pancreatectomy.
6	50			22	7		1.00			0.473	-0.018	
7	50			22	10		1.43			0.442	0.013	
8	50			22	15		2.14			0.468	-0.013	
9	50			22	20		2.85			0.477	-0.022	

we must conclude that the trouble lies neither in the production nor in the activity of the enzyme, but in some other cellular function.

MacLeod¹⁰ thinks that the influence of the ductless glands on sugar metabolism is exerted mainly on the glycogenolytic process and that there is nothing to indicate that the glycogenolytic activities undergo a change independent of accompanying glycolytic changes. We believe both of these processes, while they may be enzymic in nature, are probably for the most part carried on normally in the body by *cellular activity*, and there is some reason for considering them entirely distinct processes. In favor of the view we submit the following:

Epinephrin added either to sugar solutions containing yeast, to starch solutions containing ptyalin, or to glycogen solutions containing either ptyalin or liver extract, has no influence whatever on the diastatic activity in any of these solutions. Yet epinephrin injected into the portal vein causes an increase in the sugar content of the blood. It is known that adrenalin first stimulates, then depresses nervous tissue and perhaps other tissues also. In the liver it is known to cause inflammatory changes. It seems probable, therefore, that the glycogenolytic action of epinephrin is on the cell, since it does not influence the enzyme. Again, while chloroform and toluol greatly inhibit or stop glycolytic action, they have a much smaller action on the diastatic action of any of the above enzymes. This theory might necessitate the opinion that the glycogen exists in the cell in combination with the proteins; but this does not exclude the fact that some of it may be free, and it is due perhaps to the presence of both forms that a sharp distinction between cellular and enzymic action cannot be drawn. Pflüger's¹¹ method for the determination of glycogen, we think, indicates a protein-glycogen combination. For, if only a weak solution of KOH be used to break up the combination, the total glycogen cannot be obtained. Stronger solutions of KOH disintegrate the proteins and so liberate the combined glycogen. The action of calcium inhibiting glycosuria also indicates such a combination.¹²

In support of the cellular theory of both processes, there is a great reduction in the glycogen content of, and an increased utilization by,

¹⁰ MACLEOD: this Journal, 1912, xxix, p. 420.

¹¹ PFLÜGER: Archiv für die gesammte Physiologie, 1909, cxxix, p. 362.

¹² MCGUIGAN and BROOKS: this Journal, 1907, xviii, p. 256.

the muscles when caused to contract vigorously by either electrical stimulation, strychnine spasms, or cold. There is no evidence that enzymes are increased in rate or amount by any of these agents. Further, Rona and Doblin¹³ give evidence in favor of the view that

TABLE III.

DOGS WERE BLED ASEPTICALLY FROM THE CAROTID; THE BLOOD WAS DEFIBRINATED AND PUT INTO STERILE FLASKS, PREPARED AS INDICATED IN THE TABLE AND INCUBATED AT 40° C. AT VARYING INTERVALS 25 OR 50 C.C. OF BLOOD WAS WITHDRAWN FOR SUGAR DETERMINATION BY THE PAVY METHOD.

Flask.	Contents were defibrinated blood and	Per cent sugar content.				
		At beginning.	After 1 hr.	After 4 hr.	After 24 hr.	After 30 hr.
A21	.1600
B	Dextrose added . .	.96	.86	.76	.36	.00
....	After 2½ hr.	After 5½ hr.	After 7 hr.	After 24 hr.
C15	Trace
D	Dextrose added . .	1.25	1.07	.96	.86	.57
E	Chloroform1515
F	Dextrose and chloroform	1.25	1.25

TABLE III continued.

Flask.	Contents were defibrinated blood and	Per cent sugar content.	
		At beginning.	After 20 hr.
G	Dextrose7	.4
H	Dextrose and ½ per cent toluol . .	.7	.7
I	Dextrose and ½ per cent CHCl ₃ .	.7	.7
J	Dextrose and ½ per cent each of CHCl ₃ and toluol7	.7
K	Dextrose and ½ per cent HCHO .	.7	.52

¹³ RONA and DOBLIN: *Biochemische Zeitschrift*, 1911, xxxii, p. 489.

the glycolysis of shed blood is due to the cellular elements contained therein. Slosse¹⁴ found that in the aseptic glycolysis of blood there is neither alcohol nor carbonic acid formed, but that lactic acid is formed as an intermediate product. Fletcher,¹⁵ however, could find no evidence of the presence of a lactic acid enzyme in minced preparations of muscles to which glucose was added when these were kept aseptic. Yet living muscle can utilize glucose and does form lactic acid. Therefore cellular action is manifestly different from enzyme action.

Since the glycolysis which occurs in drawn blood is the only undoubted glycolytic action which occurs in the body fluids, we are justified in giving more weight to it than to processes which are merely assumed to be of this nature. The evidence favors the opinion that the glycolysis in the blood is due to the survival of the cellular elements. Where there is an undoubted asepsis, the rate is more rapid at the beginning and decreases with time or at the rate with which the cells die. A sharp line of demarcation cannot be made here because a part of the cell may be able to exert glycolysis and because the entire cell may not die at the same time. Chloroform or toluol, which acts very strongly on the activity of the form elements, stops the glycolysis promptly and completely. In the same way the action of anæsthetics which cause a depression of the cell action, while they do not completely stop the glycolytic action, inhibit it sufficiently to cause a glycosuria.

At present there is no clean-cut distinction between cellular and enzyme action. It may be that cellular action is a co-ordinated and integrated enzyme action. We consider the cell, however, as more than a mere collection of enzymes; it is the manufacturer and coordinator of them. The enzyme is subordinate to, and exists for the cell. As soon as a food substance is in a form which the cell can use, a ferment would appear superfluous. Since the muscle can use glucose directly, a glycolytic enzyme would seem as unnecessary as ptyalin in the saliva of a dog. Inability to manufacture enzymes when needed or a depression in the activity of an enzyme must be regarded only as a symptom of cellular depression. No explanation,

¹⁴ SLOSSE: Archives internationales de physiologie, 1912, xi, p. 153, quoted from Chemical abstracts, 1912, March 10, p. 636.

¹⁵ FLETCHER: Journal of physiology, 1912, xliii, p. 286.

therefore, of a perverted sugar metabolism which is based only upon enzyme action can be considered fundamental.

Since no substantial evidence of the presence of glycolytic enzymes in the body has been presented, the alleged co-operative influence of pancreatic extracts on the glycolytic power of muscle extracts must be regarded as an assumption unsupported by experiment. So far as glycolysis itself is concerned, experimental facts support the opinion that whatever influence the pancreas exerts in sugar metabolism, that influence must be exerted on cellular processes which are apparently not enzymic in nature.

ON FEEDING YOUNG PUPS THE ANTERIOR LOBE OF THE PITUITARY GLAND.

By T. B. ALDRICH.

WHILE the posterior lobe of the pituitary gland has been the subject of considerable investigation, as regards its blood-pressure-raising principle, and many valuable data obtained relative to its chemical and physiological side, the anterior lobe, although much the larger and looked upon as the main secretory portion of the pituitary gland, has been studied but little, and this in spite of its undoubted intimate relation with the diseases known as acromegaly and gigantism, where the pituitary gland is often found enlarged, the anterior lobe being especially involved. That the pituitary gland is associated with the growth of the skeleton and connective tissue, either alone or (what is more probable) *in association with some other glands*, is believed by all who have given the subject any thought, and this supposed relation of the pituitary body to growth, especially of young animals, has led to the employment of this gland in several feeding experiments. Prior to Schäfer's work (1909) the observations along this line, presumably carried out with the whole gland by Cerletti,¹ and of Sandri² may be mentioned.

Cerletti injected young animals (intraperitoneally) with a pituitary emulsion. His results appear to indicate *a retardation in the growth of the bones*, at any rate in the direction of length. Sandri fed young mice with pituitary apparently with the simple crude gland to the exclusion of other food. He states that this caused *an arrest of the growth*, but he had no controls. Sandri also injected young guinea pigs (intraperitoneally) with an emulsion of the gland, and observed some *apparent thickening of the bones, and atheromatous patches in the arteries*.

¹ CERLETTI: Rendiconti della reale academia dei Lincei, 1906, and 1908; also Archives italiennes de biologie, 1907, xlvii, pp. 123-134.

² SANDRI: Rivista di pathologia nervosa e mentale, 1907, xii.

Cushing³ failed to produce any constant disturbances except a *definite loss of weight* by repeated injections of extracts of pars anterior alone, using the boiled extract or the emulsion of fresh and aseptically removed glands.

Schäfer⁴ was the first to feed the two parts of this gland separately to animals: the anterior lobe for the purpose of studying its effect on the growth of young animals, the posterior lobe to study its diuretic action. In his feeding experiments with the anterior lobe, in which we are particularly interested, white rats, both young and adult, were employed. These rats were kept three or four in a cage, with an equal number of controls, usually of the same litter, in a second cage. Their food consisted of bread and milk in a certain constant proportion made into the consistency of a thick paste. The pituitary-fed rats received in addition to their regular food a small but constant amount of pituitary substance (anterior lobe), while the controls received in place of the pituitary substance an equal amount of the dried powdered substance of some other gland, usually testicle or ovary. The weight of the rats was regularly recorded.

Schäfer's experiments would lead us to conclude that the addition of a small amount of anterior lobe substance to the food favors the growth of young rats; at least it does not appear to impede or to restrict their growth.

EXPERIMENTAL.

In general the experiments here reported were carried out in a manner similar to the above, young pups, however, being used in place of rats.

These pups, seven in number (five females, two males) and from the same litter, were taken from the mother as soon as weaned, weighed, separated into two groups, each containing one male, A and B (controls), and fed on bread and milk (the bread and milk were always mixed in the proportion 1 : 2) only for eight days, from May 18 to May 26. At the end of this time they were reweighed, and each pup in Group A received daily, in addition to his bread and milk diet, 50 to 75 mg. of the fresh desiccated, defatted anterior lobe of the pituitary

³ CUSHING: *Journal of the American Medical Association*, 1909, liii, p. 250.

⁴ SCHÄFER: *Proceedings of the Royal Society*, 1909, lxxxī, p. 442.

gland of the ox in capsules; each in the other group received an equal amount of desiccated, defatted testicle or ovary, also in capsules. Desiccated ovary or testicle was given to the controls in order to conform to the conditions employed by Professor Schäfer in his feeding experiments with white rats, and to give the control dogs an equal advantage as far as any benefit is to be gained by feeding glandular

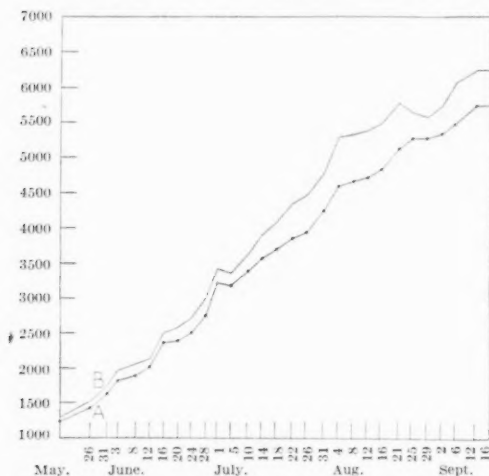


Chart showing rate of growth of the two groups of dogs. Group A, dotted line, received the addition of anterior lobe of pituitary to food; Group B, plain line, the controls received desiccated ovary. Addition was made May 26. The dates are marked upon the abscissa at proportionate intervals, while the weights are given as ordinates.

start with than Group A (45 gm.). At the end of eight days, with the same food, the dogs in Group B averaged 1498 gm. against 1411 gm. for Group A, or an average difference of 87 gm. in favor of Group B. From this it would seem as though Group B had a natural tendency to increase faster than Group A.

Although at the commencement of the experiment the pups weighed nearly the same, they fell very soon (a little over a week) into two divisions (table and chart showing this, omitted), a heavy and a light weight group, and there they remained until the end of the observations. In each group we find a pituitary-fed pup the heaviest;

tissue as such. According to Professor Schäfer the amount of calcium in the three glandular tissues given is approximately the same; if anything, the excess is against the pituitary tissue. The pups were fed twice a day in such a manner as to assure each one receiving plenty, and weighed usually every fourth day.

The results obtained are recorded in the table and chart.

It will be seen from the table (page 355) that Group B contained slightly heavier dogs to

in the group of heavy pups, 850 gm. heavier than the nearest control in this division; in the other group 70 gm. heavier than the nearest control. However, as divided, the average weight of the controls is higher than the pituitary-fed pups (456 gm.), and had the control

TABLE I.

Table giving the total weight of the two groups, A and B, together with the average weight of the pups in each group on May 18 when weaned, on May 26 when the addition of anterior lobe or ovarian (desiccated) was made, and also their weights every fourth day thereafter (with two or three exceptions) for a period of over three months. The last column shows the average increase of the controls over those fed with anterior lobe.

Date.	Group A. Pituitary fed (4 dogs, 1 male, 3 females).		Group B. Controls (3 dogs, 1 male, 2 females).		Excess of ave. weight of Group B over that of Group A.
	Total weight of animals.	Average weight.	Total weight of animals.	Average weight.	
	gm.	gm.	gm.	gm.	
May 18	4900	1225	3811	1270	45
" 26	5644	1411	4494	1498	87 ²
" 31	6428	1607	5210	1737	130
June 3	7179	1795	5865	1955	160
" 8	7518	1879	6120	2040	160
" 12	8014	2003	6351	2117	113
" 16	9515	2379	7576	2525	116
" 20	9559	2390	7728	2576	186
" 24	9968	2492	8102	2701	209
" 28	11026	2756	8961	2987	230
July 1	12901	3225	10253	3418	193
" 5	12680	3170	10070	3357	187
" 10	13427	3375	10839	3613	238
" 14	14330	3582	11780	3927	344
" 18	14765	3691	12260	4087	396
" 22	15460	3865	13000	4333	468
" 26	15820	3955	13400	4467	512
" 31	16970	4243	14340	4780	537
Aug. 4	18460	4615	15890	5297	682
" 8	18725	4681	15955	5308	627
" 12	18905	4726	16105	5368	642
" 16	19340	4835	16440	5480	645
" 21 ¹	20450	5112	17340	5780	667
" 25	20975	5244	11300	5650	406 ³
" 29	20995	5249	11170	5585	336
Sept. 2	21270	5317	11485	5742	425
" 6	21960	5490	12100	6050	560
" 12	23015	5754	12435	6217	463
" 16	23075	5769	12450	6225	456

¹ One control died. ² Tendency to increase faster. ³ Ave. for 2 dogs. One dead.

that died August 21 lived, this advantage would have been still more pronounced.

All the pups were affected approximately the same by meteorological conditions, the loss in weight from July 1 to 5 being very pronounced. It was during this period that we experienced exceedingly hot weather, the maximum temperature being 110° F. In general the curves indicate even to the end a healthy litter of pups. It is interesting to note that, although the pups were not full grown at the end of the observations, their average weight was 450-900 gm. less than their mother's (6750 gm.) and slightly above their father's weight (5400 gm.). Three of the heavier dogs, two in the control group, were 450-950 gm. heavier than their mother and 1000-1900 gm. heavier than their father. The above is explained, no doubt, in part at least, by the care and the feeding they received during this period.

From the foregoing table of average weights the following calculations have also been made:

Increase in weight in per cent of original weight:

	Pituitary.	Controls.
First week (normal diet) .	15 %	18 %
Aug. 21	317 %	355 %
Whole period	371 %	390 %

Increase in weight in per cent of weight on May 26, when feeding of glands was commenced:

	Pituitary.	Controls.
First week (May 26-June 3)	27 %	30 %
Aug. 21.	262 %	286 %
Whole period	309 %	316 %

These percentages show that the controls grew more than the others; but they also show that the *rate of increase* in the pituitary group increased faster than in the control group.

One fact stands out prominently, that given in this amount neither the anterior lobe nor desiccated ovary arrested the growth of young pups.

Furthermore, had the pups by chance been divided differently, the most striking results would have been obtained, all referable to

the effect of feeding the anterior lobe. This only goes to emphasize the importance of making a number of feeding experiments before drawing conclusions. Schäfer found that the addition of the anterior lobe of the pituitary gland to the food of young rats stimulated their growth. My experiments on dogs seem to show the opposite effect, or at least to show that its average effect is below that of the controls, at the same time intimating that the growth of certain individuals may be stimulated, since a pituitary-fed dog was the heaviest (850 gm. heavier than its nearest control). Before we can arrive at any positive conclusions relative to this question, it will be necessary to experiment further, not only on rats and dogs, but on other animals. At the present time feeding experiments are being carried out with white rats. These results will be reported in a later paper.

CONCLUSIONS.

1. The daily addition of 50-75 mg. of fresh desiccated, defatted anterior lobe of the ox to the food of young dogs does not stimulate in general their growth, as evidenced by their weight.
2. The growth of young dogs is certainly not retarded or impeded by the addition of the desiccated, anterior lobe, ovary or testicle to their food.
3. In some individual instances the addition of the anterior lobe may stimulate growth.

THE RELATIONSHIP OF THE SINO-AURICULAR NODE TO AURICULAR RHYTHMICITY.

By V. H. K. MOORHOUSE.

[From the Physiological Laboratory of Washington University, St. Louis.]

INTRODUCTION.

THE significance of the sino-auricular node as regards cardiac rhythmicity has been the subject of extensive physiological and pathological research since the discovery of the specialized tissue by Keith and Flack¹ and its more detailed description by Koch.² This report has special reference to the sino-auricular node of the dog's heart, and a brief description of the extent and character of the nodal tissue of that animal is therefore important. The node, as described by Koch, occupies a position along the sulcus terminalis beginning just below the border between the superior vena cava and the right auricular appendix and extending for a variable distance (1-3 cm.) in the sulcus terminalis towards the inferior vena cava. In form the node is spindle- or club-shaped with its thickest portion (the head) placed superiorly and its stem tapering off into a fine inferior process. A superior process is described extending from the head for a short distance over the cava-appendicular angle. In histological structure the muscular elements are of an embryonic type and are invested by greater or less amounts of connective tissue. The node shows intimate connections with nervous tissues, nerve fibres and ganglion cells being scattered throughout its structure. The muscle elements pass over imperceptibly into the muscular tissue surrounding. A well-marked artery runs in the sulcus and is in close association with the nodal tissue, supplying many branches to it and to the surrounding musculature. For a more detailed description of the node reference may be made to the article of Keith and Flack,¹ and Koch.²

¹ KEITH and FLACK: *Journal of anatomy and physiology*, 1907, xli, p. 172.

² KOCH: *Medizinische Klinik*, 1911-12.

Physiological experiment has concerned itself with the effect of removal of the nodal area in the intact and perfused heart. From the above description of the relations of the node, it will be seen how impossible it is to remove the specialized tissue without doing damage to other structures. Any conclusions, therefore, from the result of such removal must be provisional. Flack,³ working on intact dogs' hearts, removed the nodal tissue by clamp and ligature. He states that in no case did such isolation have any marked effect on heart rate. Jäger⁴ destroyed the nodal tissue by thermal cautery in intact dogs' hearts. He carried out histological examination and states that destruction of the nodal tissue had no effect on auricular rate. Removal of the area containing the node in perfused hearts has been carried out by many observers before and since the discovery of the specialized tissue. Langendorff and Lehmann⁵ found that the Stannius experiment upon excised cats' and rabbits' hearts resulted in stoppage with subsequent slowing of the auricles and ventricles. On the other hand, Erlanger and Blackmann⁶ have shown that this is not always the case. From a consideration of their protocols it is seen that in some cases extensive removal of areas at the mouths of the great veins did not cause change in auricular rhythm. Though their work did not have special reference to the sino-auricular node, the possibility that in such cases there remained some specialized tissue to account for the unaffected rate is absolutely excluded by the position and extent of the tissue removed. Lohmann⁷ applied formol to the region of the node and notes a marked slowing of rate as the result. The difficulty of localizing this effect is apparent. Cohn and Kessell,⁸ working on perfused dogs' hearts, state that removal of a rectangular area containing the node causes in all cases stoppage and subsequent slowing of auricular rate, which never regains its former rapidity. They conclude that no other area is capable of taking up the function of pacemaker. Their details of experiments and histological examination are still

³ FLACK: *Archives internationales de physiologie*, 1911, xi, p. 111.

⁴ JÄGER: *Deutsches Archiv für klinische Medizin*, 1910, c, p. 1.

⁵ LANGENDORFF and LEHMANN: *Archiv für die gesammte Physiologie*, 1906, cxii, p. 352.

⁶ ERLANGER and BLACKMANN: *this Journal*, 1907, xix, p. 125.

⁷ LOHMANN: *Archiv für die gesammte Physiologie*, 1908, cxxiii, p. 628.

⁸ COHN and KESSELL: *Archives of internal medicine*, 1911, vii, p. 226.

unpublished. A very different conclusion is arrived at by Magnus-Alsleben,⁹ who removed the nodal tissue, together with extensive areas from the right auricle of rabbits' hearts. He concludes that no specific centre exists at the junction of the superior vena cava with the right auricle which is especially capable of impulse production. He notes that the sinus node region possesses a relative superiority, because shutting off its influence may lead to short periods of irregularity, but states that after such removal other areas may take up autonomy without change in rate.

The following series of experiments was undertaken with the object of testing in various ways the specificity of the sino-auricular node for the determination of auricular beat.

METHOD.

Dogs' hearts were excised with the usual precautions and perfused with oxygenated Locke's solution at a constant pressure (60-70 mm. Hg) and temperature (35-36° C.). The suspension method was used and the right auricular contractions were recorded by air transmission. The portions of tissue removed were fixed in formalin, and in those experiments where histological examination was carried out, imbedded in paraffin. Sections were cut at right angles to the sulcus terminalis and stained with hæmatoxylin and picroacid fuchsin. Where series of sections were cut, every twentieth section was mounted in balsam.

OBSERVATIONS.

I. As soon as the heart assumed a regular rate cuts were made in the sinus region in various ways:

(a) A rectangular area, presumably containing the sino-auricular node, was removed by a series of four cuts. The position of this area A is shown in the accompanying diagram (Fig. 1). The length of the strip varied from 1 to 3 cm., depending on the size of the heart. Removal of this area A gave results as follows:

i. Stoppage of the auricles and subsequent taking up of a rate slower than before the cuts.

⁹ MAGNUS-ALSLEBEN: *Archiv für experimentelle Pathologie und Pharmakologie*, 1911, lxiv, p. 228.

- ii. Gradual slowing of auricle without stoppage.
- iii. Auricles proceed at former rate without interruption (see Table I).

Stoppage of the auricles as a result of operative procedures is not especially significant, since during perfusion experiments it may be elicited by various mechanical stimuli. Erlanger⁶ has pointed out that a mere touch to the auricle in perfusion experiments may result in stoppage. Frequently one sees a single cut causing standstill for short periods. The condition has been explained as a form of shock.

In cases of the third type it was, of course, necessary to eliminate any possibility of the presence of nodal prolongations. Therefore, after removal of the area A, the cuts were extended towards the inferior vena cava and the area B (Fig. 1) was removed. In some experiments this was sufficient to cause stoppage of the auricles with production of a slower rhythm, but in others it was very evident that the impulse was not proceeding from nodal tissue, since control removal of the sinus region from the inferior vena cava to the auriculo-ventricular boundary above the cava appendicular angle did not influence the rate. In these cases subsequent removal of other highly rhythmical portions of the sinus region caused stoppage and slowing of the auricle.

(b) Removal of an area B (Fig. 1) was next carried out, as soon as the heart was beating regularly. Area B is a rectangular strip from the lower part of the sinus region, including the tissue adjacent to the mouth of the inferior vena cava, and the lower part of the sulcus terminalis. In length this strip was about 1 cm. Removal of this area B

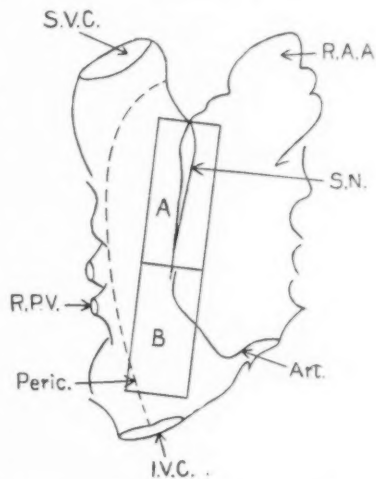


FIGURE 1.—Diagram of right auricle, showing position of areas A and B.—S.V.C., superior vena cava; I.V.C., inferior vena cava; R.A.A., right auricular appendix; Peric., pericardial attachment; R.P.V., right pulmonary vein; Art., artery to sinus region; S.N., sinus node.

TABLE I.

Experiment No.	Rate in 10" before operation.	Isolation of Area A.	Rate after operation. ¹	Isolation of Area B.	Rate after operation.	Remarks.
1	15	Cut	S. 11
2	15	Cut	S. 12
5	19	Cut	20	Twenty minutes later auricle rate slows to 15.
6	15	Cut	S. 10
7	21	Cut	21	Cut	S. 8	{ Subsequent removal of B causing definite slowing.
8	14	Cut	S. 7	{ Histol. Exam. Sections from area B show no nodal tissue.
9	15	Cut	9	{ Gradual slowing in fifteen min. Histol. No well-marked nodal prolongations.
10	14	Cut	10	{ Slowing in two min. Histol. No well-marked nodal tissue.
11	11	Cut	11	{ Histol. Fine processes of nodal tissue.
13	20	Cut	S. 10
14	14	Cut	14	Cut	S. 8	Histol. Sections from area B, no nodal tissue.
21	21	Clamp	18	Subsequent cuts cause gradual slowing to 10.
23	14	Clamp	S. 6
24	21	Clamp	S. 16	{ Removal by cuts causes further gradual slowing.
25	18	Clamp	12

26	17	Clamp	S. 9	Rate not much affected by extensive removal. Subsequent cut disturbing blood supply to B causes stoppage. Histol. Area A contains S. A. node.
30	12	Clamped, then cut	10	
32	16	Cut	17	
33	15	Cut	14	S. 4	
35	17	Clamp	S. 12	
36	16	Clamp	S. 6	
39	15	Clamp	S. 6	
40	12	Clamp	10	
41	15	
42	14	Clamp	S. 3	
3	16	Cut	15	13	
4	20	Cut	19	15	
12	16	Cut	16	18	
					17	
22	19	Clamp	19	
48	15	Cut	15	
50	15	Clamp	15	15	
					14	

Very slight effect of complete isolation.

Subsequent removal of both areas by cuts, auricle slows to 10.
Removed tissue in cava-appendicular angle.
Removal of tissue in coronary region — rate slows to 13.
Later — single cut to coronary sinus, S. 7.
Clamp to mesial wall of superior vena cava, 13. Histol. exam. of Area A, well marked S. A. node.
Later clamped area removed and cava-appendicular region excised. Rate after this, 17. Removal of inferior caval area of septum — S. 6.
Single cut on mesial border of superior vena cava causes stoppage.
Whole clamped area removed. Cava-appendicular tissue excised. Rate after this, 13.
Inferior caval area of septum excised, S. 6.

¹ The rates given after the operations are in all cases the maximal rate of auricle after a period had been allowed for disappearance of any mechanical stimulatory effects.

² S. means auricle stopped for a variable period, 2'-60".

by cuts gave results practically identical with those obtained from removal of area A, *viz.*, stoppage and slowing, gradual slowing or no interruption of auricular rate (see Table I). Where the auricle continued to beat uninterruptedly, the cuts were extended upwards and the area of tissue A containing the nodal tissue was removed. In some cases this sufficed to slow auricular rate, but in others removal of the whole area occupied by the specialized tissue was without effect on the rhythm.

Histological examination of the area B, as seen in Table I, showed that no extensions of the nodal tissue were to be found, or, if present, were very small and identified only with difficulty.

II. The greatest objection to the removal by cutting of an area of tissue from the sulcus terminalis is that the artery supplying an extensive area of highly rhythmical tissue is severed. Thus removal of the area B interrupts the perfusion fluid supply to the area A. The same objection applies to the removal of area A, since the fluid pressure throughout the capillary area of the lower part is reduced, and the condition of the muscle suffers accordingly. In the case of the area A the fluid supply is often well kept up by the anastomotic circle around the superior vena cava. The gradual slowing of auricular rate observed in some of the experiments is safely attributable to the disturbance of fluid supply. The fact that the auricle does not in many cases resume its former rate after removal of either area may also be caused by disturbance of the fluid supply to muscle tissue slightly impaired by the artificial conditions of perfusion experiments. Experiments were carried out in which the area was first clamped and then removed by cuts, and frequently the subsequent cuts caused a gradual slowing of rate, *e. g.*, Exps. 21 and 24, Table I. For these reasons isolation was afterwards carried out by means of a rectangular clamp which could be inserted through an incision without interfering with the perfusion fluid supply for more than forty to fifty seconds. The clamp causes complete functional isolation of the area enclosed. Clamping experiments, as seen in Table I, gave a series of results similar to those obtained in the cutting experiments. Clamping off the area B was just as efficient in causing stoppage and slowing of auricle as removal of area A.

Experiments were undertaken with the object of comparing the rates of the two areas A and B. For this purpose the whole area

(A B, Fig. 1) was isolated without disturbing its fluid supply. Then this area was divided into two sections functionally by clamping across near the centre. The two halves were compared as to rate by counting with a stop watch and in some case tracings were obtained. The area containing the node exhibited no constant predominance of rate in these experiments. In Experiment 43, where the rates of the two areas were equal, series of sections carried through both areas showed that the nodal tissue was relegated to the area A. In Experiment 44, where the rate assumed by A was distinctly slower than that of B, serial sections showed that the nodal tissue was confined to the area possessing the slower rhythm. The results from all the experiments of this type lead one to think that there is a balance of power throughout this portion of the sinus region in the matter of rhythmicity.

The six experiments quoted at the end of Table I clearly controvert the specificity of the node for cardiac rate. In these experiments all the node-bearing area was removed, together with all parts possibly containing prolongations of nodal tissue, and extensive amounts of caval, sinus, and auricular musculature. A detailed protocol of an experiment is illustrative of the procedure and result:

Experiment 38. Dog. December 12, 1911. —

Rate of auricle in ten seconds at commencement	15
After removal of area A	14
After removal of area B	14
Tissue around superior cava and in cava-appendicular region crushed with clamp. After this procedure	14
Cut removing area from auricular septum at mouth of right pulmonary vein. Auricle stops — later rate resumed	9

Histological examination. — Sections from the area removed from auricular septum at mouth of pulmonary vein contain no trace of nodal tissue.

The function of determination of auricular beat was assumed without pause by some other highly rhythmical area. The musculature of the coronary sinus, auricular septum, or superior vena cava was probably concerned in this determination of beat, since removal of the influence of these areas was effective in slowing auricular rate (see Table I). These regions are developmental parts of the sinus, and their

high grade of rhythmicity has been pointed out by Erlanger.¹⁰ Histological examination verified the justifiable conclusion that the nodal tissue had been removed.

DISCUSSION.

The assumption that the sino-auricular node is of paramount importance in determining cardiac rhythm is not consistently borne out by experimental evidence. In perfusion experiments removal of the area containing the specialized tissue is frequently followed by

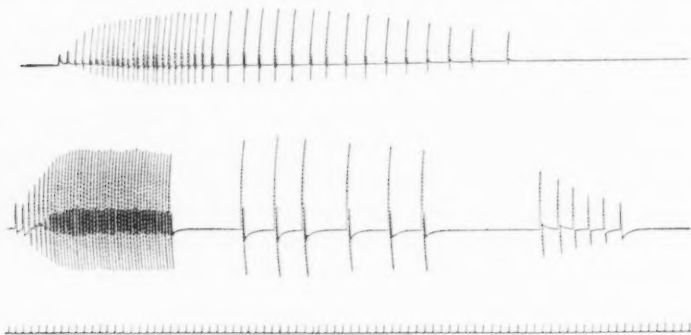


FIGURE 2. — Four fifths the original size. The upper tracing is from the strip S.A. containing the sinus node, and the middle from control strip S.B. Both stimulated through and through and immersed in Ringer-Locke fluid. Time in 1 sec. intervals.

disturbances in rate, but if the specificity of the nodal tissue is to be upheld, uniformly positive results are necessary. Clear negative results absolutely disprove the specificity of the nodal tissue as regards cardiac rhythm. Removal of the nodal tissue cannot be carried out without injury to the important structures around. Disturbances in rate in perfusion experiments may therefore be caused not by removal of the nodal tissue, but of the surrounding musculature. Excision of an area not containing nodal tissue has been shown to cause definite disturbance in rate. When comparison as to rhythmicity is made between the node-bearing region and an area not containing nodal tissue, no striking difference has been observed. For these

¹⁰ ERLANGER: this Journal, 1910, xxvii, p. 87.

reasons the attempt to correlate specific histological structure with specific physiological property has failed. From other points of view the proof of specificity of the sino-auricular node is not conclusive. Lewis,¹¹ by locating the point of primary negativity by means of the string galvanometer, does not exhaust the possibilities. His points of contact are on the superficies of the auricle, leaving out of account the highly rhythmical, more deeply placed areas. Among his results are evidences indicating that the place of origin of the beat was not uniform, *e. g.*, in Experiment 3, the point P₂ was primarily negative to the point T₂. The similar work of Wybauw¹² locates the point of primary negativity farther down on the sulcus terminalis than the point T₂ of Lewis. A study of the pathological literature shows us that the question is undecided. Cohn¹³ reports a case of bradycardia with definite sclerosis and infiltration of the sinus node as the outstanding feature. It is to be pointed out that it is not proven experimentally that destruction of the node causes bradycardia. Draper¹⁴ reports a case of *pulsus irregularis perpetuus* with fibrosis of the sinus node and artery. It has been demonstrated that auricular fibrillation may result from stimulation of any part of the auricle. Koch¹⁵ is inclined to attribute the changes in rhythm in certain cases not to inflammation or destruction of the sinus node, but to extension to and infiltration of the surrounding tissues. Flack¹⁶ points out that, on the basis of experimental work, destruction of the node by pathological processes may only play a mediocre rôle in the development of irregu-

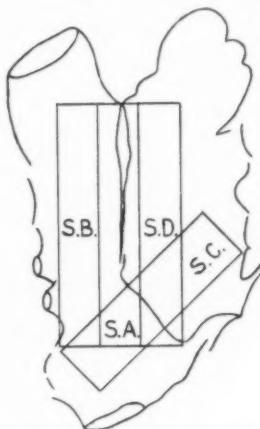


FIGURE 3.—Diagram of right auricle, showing position of various strips, whose rhythmicity was compared with that of the strip containing the sinus node (S.A.)

¹¹ LEWIS: Heart, 1910, ii.

¹² WYBAUW: Archives internationales de physiologie, 1910, x.

¹³ COHN: Heart, 1911, iii, p. 23.

¹⁴ DRAPER: Heart, 1911, iii, p. 13.

¹⁵ KOCH: Verhandlungen der deutschen pathologischen Gesellschaft, 1909-10, p. 85.

¹⁶ FLACK: Archives internationales de physiologie, 1911, xi, p. 127.

larities. Sufficient pathological evidence has not as yet accumulated to throw much light on the problem.

Since the completion of the above series of experiments confirmation has been achieved in a very striking manner by another method. The rhythmicity of an auricular strip containing the sinus node has been compared with that of a strip not containing nodal tissue under exactly the same conditions. The method is that described by Erlanger,¹⁰ and consists in immersion of both strips in a bath of oxygenated Ringer solution at body temperature. The strips are connected in series and stimulated through and through with exactly the same induced current. The accompanying tracing (Fig. 2) is one of a series obtained from auricular strips of the dog's and cat's heart. This tracing shows clearly that the rhythmicity of the strip containing the sinus node (S. A.) was less than that of the strip (S. B.) with which it was compared. The positions of S. A. and S. B. are shown in the diagram (Fig. 3). Other strips (S. C. and S. D.) have also been compared with S. A., and the results conclusively show that the sinus node is far from exhibiting a constant predominance of rate.

SUMMARY.

From the results of the perfusion experiments here reported it cannot be concluded that the sino-auricular node is specific as regards the determination of cardiac rate.

The rhythmicity of the musculature of the sinus region is shown to be in no way exceeded by that of the specialized tissue.

After removal of the nodal tissue other areas not containing specialized musculature are quite capable of maintaining the normal rate of the heart.

This work was taken up at the suggestion of Dr. Erlanger, to whom my best thanks are due for his valuable advice and help. I am also indebted to Dr. Terry and Dr. Emmel for their kind assistance with the histological work.

THE RESPONSE OF THE VASOMOTOR CENTRE TO DEPRESSOR STIMULATION.

BY TORALD SOLLMANN AND J. D. PILCHER.

[From the Pharmacological Laboratory of the Medical School of Western Reserve University,
Cleveland.]

I. REACTIONS OF THE VASOMOTOR CENTRE.

WE have confined our investigations mainly to cats, since rabbits were not promising subjects for our perfusion method of studying central vasomotor reactions. It is well known that the depressor nerve in the cat runs in the cervical vagus trunk, where it evidently predominates over the vasoconstrictor fibres. This was the case in all our animals.

In performing the experiments the cats were anaesthetized, usually by morphine-atropin-ethylcarbamate, and generally curarized. Both vagi were divided, and the central stumps prepared for stimulation. Oxygen insufflation or artificial respiration was applied as needed. The spleen or the kidney or the iliac vessels were prepared for perfusion, according to the method described in our first paper.¹ A quickening of the perfusion flow indicates central vasodilation and *vice versa*.

The spleen (ten animals) and the kidneys (three animals) always responded to the depressor stimulation by an unmistakable dilatation (Fig. 1). The average of eleven typical animals shows a fall of blood pressure from 119 to 78 = 41 mm., with 17 per cent increase in the perfusion flow. Very exceptionally, individual stimulations showed apparent vasoconstriction, and this even when the blood pressure was lowered.

Sometimes the flow would show a transitory preliminary slowing (as in Fig. 3). Since the blood pressure did not rise, this is evidently due to the adjustment of the perfusion fluid to the increased vascular

¹ SOLLMANN and PILCHER: this Journal, 1910, xxvi, pp. 233-238.

area. Exceptionally the flow was temporarily slowed after removing the stimulation (C 103). This must be attributed to vasoconstriction,

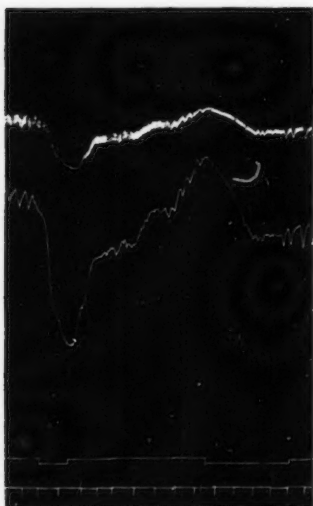


FIGURE 1. — One half the original size. Depressor stimulation. The cat (C 76.1) had been atropinized and both vagi divided, oxygen insufflation. Upper tracing, membrane manometer; second tracing, damped mercury manometer, both in carotid artery. Lowest line, flow from perfused spleen. The left vagus depressor was stimulated at the left signal, the right at the right signal. Note the quickening of the perfusion flow with the fall of pressure.

either through vasoconstrictor fibres in the vagus or, more probably, through cerebral anemia.

The iliac perfusions (two animals) were not decisive, since the depressor response was weak. Only one stimulation showed a typical fall of pressure, and this also showed an increased perfusion flow. This is in line with the statement of Bayliss² that the leg participates in the vasodilation.

Effect on the pulse pressure. — The carotid tracings taken with a membrane manometer show a conspicuous weakening of the blood pressure beats (Figs. 1, 3, and 4) in most but not all of the animals. This decrease of the pulse pressure might be due either to weakened contractions or to lessened resistance.

Comparative effect of stimulation of the right and left nerves, and of bilateral stimulation. — It is stated by Bayliss³ that the left depressor (in rabbits) is usually the more effective, and that stimulation of both depressors gives a further fall. Of five cats which we ob-

served in this respect, in three (C 99, 102, and 208) both sides elicited exactly the same response; in one (C 102) the right caused a slightly greater fall, and in another (C 76) the left was considerably more active. The quickening of the perfusion flow averaged higher with right stimulation, but this is probably accidental. The pulse pressure

² BAYLISS: *Journal of physiology*, 1893, xiv, p. 303.

³ BAYLISS: *Loc. cit.*, p. 314.

changes were generally the same from both sides. The differences in the two sides are therefore inconstant.

Simultaneous stimulation of both nerves produced but very little greater effect than stimulation of the more active nerve alone.

Strength of stimuli. — It was found that the blood pressure fall increased gradually with the strength of the inductive current, from the minimal effective strength until the maximum response was reached.

Our current strength was not measured with accuracy, so that the data are not available for confirming the relation curve of Porter.⁴ Vasoconstrictor effects could not be evoked by modifying the strength of the stimulation.

II. RELATION OF THE DEPRESSOR FALL TO THE LEVEL OF BLOOD PRESSURE.

In a previous paper⁵ we have emphasized that cardiovascular reactions in general are modified by the level of blood pressure at which they are produced. The present investigation furnished the opportunity of collecting a fair number of data on vagus-depressor stimulation in cats. These have been grouped after the manner suggested by Porter,⁶ but separating the blood pressures by 20 mm. Both vagi were divided, and the cephalic end of one was stimulated with induction shocks. The strength of the shocks was adjusted so as to give a practically maximal response, and in most of the animals several stimulations were made at different levels, so that we believe the results to be fairly representative for cats. The smoothness of the curves argues for their accuracy. Nineteen animals were used. The results are shown in Table I.

In Fig. 2 the results have been plotted as curves, together with the equivalent data of Porter⁴ on rabbits. It will be noted that the curves from the two animals are quite different.

With rabbits the absolute fall of pressure is nearly the same with pressures between 75 and 145 mm.; in cats the absolute fall increases

⁴ PORTER: this Journal, 1910, xxvii, p. 276.

⁵ SOLLMANN and PILCHER: Journal of pharmacology and experimental therapeutics, 1911, iii, p. 48.

⁶ PORTER: this Journal, 1907, xx, p. 403.

very conspicuously from 80 to 160 mm., so that the pressure in the trough of the fall is almost the same whether the original level was 100 mm. or 160 mm. The percentile fall in the rabbit increases to the

level of 85 mm., and decreases progressively at higher levels. In the cat it increases steadily to 175 mm.

The difference may be due to the fact that constrictor fibres are also stimulated in the cat; and these constrictor impulses may be less effective when the level of blood pressure is high. However, this is mere speculation. More likely the greater fall from the higher blood pressure is cardiac, the heart being more sensitive to disturbances of its blood supply. In support of this these large falls are generally (but not always) accompanied by diminished pulse pressure (Figs. 1, 3, and 4).

The main importance of the curve, for our present purpose, is its empirical use as a base-line for judging the influence of various experimental procedures on the depressor response.

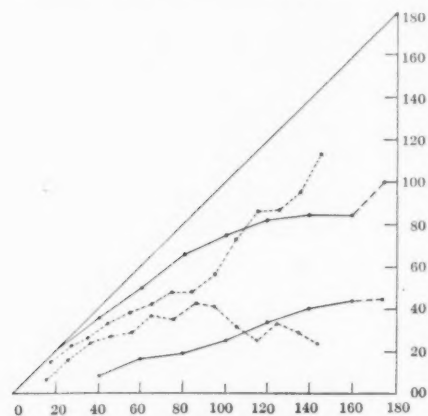


FIGURE 2.—Depressor response from different levels of blood pressure. The diagonal indicates the ascending levels of blood pressure at which the stimulations were made. The two upper curves give the average stand of pressure during the stimulation. The vertical distance between this and the diagonal shows the absolute fall of blood pressure. The percentile fall corresponds to the distance between the base-line and the lower two curves. The marginal numbers indicate the level of blood pressure in millimetres, and the percentage for the percentile fall. The plain curves are for the cat, the dotted curves for rabbits (plotted from the data in Porter's paper).

Since the absolute fall and percentile fall are parallel, but the range of variation is smaller for the percentile fall, this latter would be the more useful criterion for depressor response in the cat.

III. DEPRESSOR STIMULATION DURING OCCLUSION OF THE AORTA.

Cyon and Ludwig,⁷ in their paper announcing the discovery of the depressor nerve in the rabbit, also tried its stimulation during compres-

⁷ CYON and LUDWIG: Arbeiten aus der physiologischen Anstalt zu Leipzig, p. 128. Cited from HENLE and MEISSNER's Jahresberichte, 1866, p. 425.

sion of the aorta to show the share of the splanchnic vessels (and of course also the vessels of the hind legs) in the fall. Occluding the aorta just below the diaphragm, they found the response to depressor stimulation greatly reduced or even abolished. Von den Velden⁸ (p. 238) reports a similar experiment, in which the response was re-

TABLE I.
RESPONSE TO DEPRESSOR STIMULATION IN CATS.

Level of blood pressure.	Number of observations.	Average absolute fall.	Average percentile fall.
mm. 176	1	mm. 76	43
151-170	5	77	42
131-150	5	56	40
111-130	7	39	33
91-110	7	25	25
71- 90	7	15	19
51- 70	8	10	17
31- 50	4	3.3	8

duced from 23 per cent to 6 per cent. He further found that the response remained absent for some time after the aorta was released, attributing this to temporary anemic paralysis of the peripheral vasomotor nerve-endings.

We made a number of experiments with intrathoracic compression of the aorta with the following results:

In two animals the depressor fall was practically abolished during aortic compression, namely:

C 102 Fall before compression, 105-70 = 35 mm.

During compression, 148-143 = 5 mm.

C 211 Fall before compression, 120-75 = 45 mm.

During compression, 140-140 = 0 mm.

⁸ VON DEN VELDEN: *Archiv für experimentelle Pathologie und Pharmacologie*, 1906, IV, p. 223.

In two animals the depressor fall was materially diminished during compression, namely:

C 211 Before compression, $110-78 = 32$ mm.

During compression, $130-110 = 20$ mm.

C 99 Before compression, $138-70 = 68$ mm.

During compression, $125-103 = 22$ mm.

In two other animals, however, the depressor fall was not impaired:

C 77 Before compression, $158-78 = 80$ mm.

During compression $150-68 = 82$ mm.

C 73 Before compression (not tried).

During compression, $176-100 = 76$ mm.

The relevant tracings for Experiment 77 are shown in Figs. 3 and 4.

The fact that the response is never increased, notwithstanding the higher blood pressure, suffices to show that the exclusion of the posterior vascular area diminished the vascular response. The considerable remaining response in individual animals might be explained by heightened response of the remaining vascular areas or by weakening of the cardiac contractions. Our perfusion experiments did not show that the excitability of the depressor centre is increased during compression, and the excitability of the peripheral vasomotor system is diminished, as shown by the diminished reaction, which persists, often for some time, after the pressure is released (for instance, in Experiment 102 the response was: before compression, $105-70 = 35$; during compression, $148-143 = 5$; immediately after release, $90-85 = 5$; twenty minutes later, $80-75 = 5$. In other experiments the difference may not be important; for instance, in C 209 the fall was, before compression, $98-70 = 28$; two minutes after compression it was $80-60 = 22$).

The large fall occasionally seen during aortic compression must therefore be due to cardiac weakening, and a glance at the tracings of Figs. 3 and 4 shows plain evidence of cardiac depression both before and during aortic compression.

IV. THE CARDIAC BEHAVIOR IN DEPRESSOR STIMULATION.

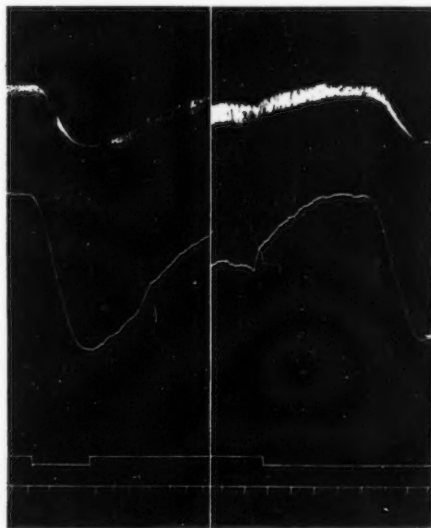
The weakening of the pulse pressure during depressor stimulation led us to examine further into the direct or indirect cardiac response.

Lehndorff⁹ appears to be the only one who has taken the cardiac factor into consideration. He states (p. 389) that the depressor fall sometimes occurs in two steps, of which the second should be attributed to cardiac failure from deficient blood supply. We have occasionally seen acute death with such two-step tracings (C73). The ordinary weakening of the pulse pressure is rather different, for it occurs early (Figs. 1 and 3); but it might still be due to interference with the blood supply, or it could be due to a nervous cardiac reflex, analogous to the carotid traction reflex described by Sollmann and Brown.¹⁰

Finally, of course, changes in the pulse pressure do not necessarily mean corresponding changes in the heart. In order to study the cardiac phenomenon we made a series of myographic tracings. Cats

were anaesthetized as usual with atropin, morphine, and ethylcarbamate, sometimes with curare and sometimes without. Both vagi were divided. In order to prevent the interference of shock the cardiac tracings were generally taken by the acupuncture method, a needle being thrust through the chest into the heart near the apex. The Cushny myocardiograph was used in one experiment.

The results were found to differ for normal and low blood pressures.



FIGURES 3 and 4. — One half the original size. Depressor stimulation before (Fig. 3) and during (Fig. 4) aortic compression (Cat C 77-2). The signal in Fig. 3 indicates the stimulation. In Fig. 4 the aorta was clamped at the first signal and the depressor stimulated at the second signal, the aorta remaining clamped. Note the weakening of the pulse pressure in both tracings.

⁹ LEHDORFF: *Archiv für Physiologie*, 1908, p. 362.

¹⁰ SOLLMANN and BROWN: *this Journal*, 1912, xxx, p. 88.

With normal blood pressure (80 to 130 mm.) the *depressor stimulation* was invariably accompanied by decrease of the cardiac volume (seven stimulations on animals C 209, 209a, 210, and 211). The decrease involved both the systolic and diastolic volume; and on the

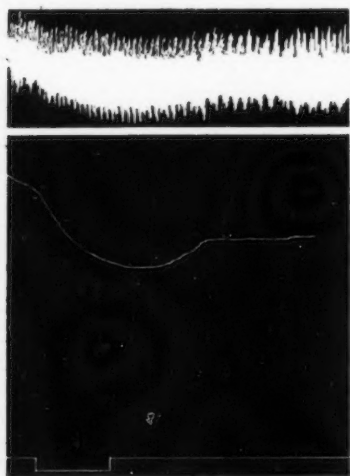


FIGURE 5.—About two thirds the original size. Cardiomyogram with depressor stimulation (Exp. C 211). The upper tracing was taken with a needle myocardiograph; the upstroke corresponds to diastole and increase of cardiac volume, and *vice versa*. The lower tracing is the carotid blood pressure. The vagus depressor was stimulated, at the signal, with strong shocks.

whole to about an equal degree, although one or the other generally showed a somewhat greater diminution (Fig. 5). The amplitudes of the individual excursions were not materially altered; on the whole, they tended to an insignificant decrease.

The diminished cardiac volume means an imperfect filling of the cardiac cavities, doubtless due to accumulation of blood in the distending vessels, and thereby its withdrawal from the effective circulation. This agrees with the statement of Bayliss¹¹ that depressor stimulation generally lowers the pressure in the vena cava.

With a moderate dilation, as in most of the experiments under consideration, this interference with the blood supply was not sufficiently serious to embarrass the heart, but when the drop of pressure was large (50 mm., Experiments 209a and 211) there were also evidences of cardiac weakening.

Again, when the blood pressure was low (40 to 60 mm., Experiments 208 and 209), the depressor stimulation showed a distinct tendency to dilate the heart. The cardiac muscle, already suffering from a faulty blood supply, is evidently more easily injured by the depressor dilatation.

We also tried the effect of stimulating the depressor during occlu-

¹¹ BAYLISS: Journal of physiology, 1902, xxviii, p. 293.

sion of the thoracic aorta. In five observations on two animals the results were qualitatively the same as the typical results with open aorta. Quantitatively they were weaker.

We may therefore conclude that the depressor stimulations do not affect the heart directly or reflexly, but that the heart is affected indirectly by the altered distribution of the blood. Ordinarily this produces only a decrease in the mean volume of the heart, leaving the amplitude of the contraction unaltered. But if the depressor effect is excessive (as under high blood pressure), or if the cardiac efficiency is already poor (as under low blood pressure), then the depressor stimulation results in further impairment of the heart.

V. THE INFLUENCE OF OTHER EXPERIMENTAL PROCEDURES UPON DEPRESSOR RESPONSE.

This has been studied, directly or incidentally, by a number of observers, most extensively perhaps by Tschirwinsky¹² and by Von den Velden.¹³ According to Von den Velden, the response is increased by those measures which cause moderate stimulation of the vasomotor centre. Strong stimulation of the vasomotor centre lessens or prevents the depressor fall. It is also prevented by peripheral vasomotor stimulation (epinephrin, digitalis). Depression of the vasomotor centre by narcotics likewise lessens the response to depressor stimulation.

In the following we shall touch only upon those measures which we have investigated personally.

Effect of strychnin on depressor stimulation.—The fact that small doses of strychnin increase the efficiency of depressor stimulation (*i. e.*, that it gives a larger fall) has been noted by Bayliss¹⁴ and by Von den Velden.¹⁵ This has also been our experience: with a dosage of 0.05 mg. per kg. the blood pressure fall was distinctly, though not greatly, increased. The perfusion flow also showed greater quickening. Our data show that this increased response is not due to increased

¹² TSCHIRWINSKY: *Centralblatt für Physiologie*, 1895, ix, p. 977.

¹³ VON DEN VELDEN: *Archiv für experimentelle Pathologie und Pharmacologie*, 1906, lv, p. 223.

¹⁴ BAYLISS: *Proceedings of the Royal Society*, 1908, lxxx, p. 357.

¹⁵ VON DEN VELDEN: *Loc. cit.*

level of blood pressure, for this happened to be slightly lowered after the strychnin.

The following are the averages from six experiments:

Depressor response (fall of pressure) before strychnin, 95 to 75 = 19 mm.
= 20 per cent.

Depressor response (fall of pressure) after strychnin, 85 to 64 = 21 mm.
= 25 per cent.

According to Bayliss¹⁴ increased response is due to increased excitability of the vasodilator centre. We did not investigate the effects of toxic doses of strychnin. Bayliss describes a gradual reversal of the depressor response into a pressor response (paralysis of the vasodilator centre, and reversal of the reciprocal inhibitory effect on the vasoconstrictors into an augmentory effect). Tschirwinsky and Von den Velden merely note that the depressor fall is weakened, and abolished in the convulsive stage. Velden states that it is ineffective during the convulsive rise, but effective during the intervening period of relative vasomotor depression.

Compression of both carotid arteries.— Von den Velden found that this emphasized the depressor response. We found (in two experiments) that it does not noticeably improve poor depressor reactions (total falls of 15 mm. and less). We did not try its effect on good depressor reactions. Von den Velden attributes the increased excitability of the dilator centre to slight cerebral anemia (but, as Bayliss¹⁶ points out, clamping the carotids would not interrupt the blood supply of the vasomotor centre, which comes chiefly from the vertebral arteries).

Traction on the carotid artery, and the consequent fall of blood pressure, does not appear to affect the depressor reaction directly,¹⁷ the depressor fall being somewhat decreased (from 33 per cent at 70 mm. to 24 per cent at 54 mm.). This reduction may be ascribed to the lower level of blood pressure, for it agrees very well with the average figures of Porter for these levels.

Effect of normal saline solution.— Porter and Beyer¹⁸ found that restoration of the blood pressure by saline injection, after section of

¹⁶ BAYLISS: *Journal of physiology*, 1893, xiv, p. 315.

¹⁷ SOLLMANN and BROWN: *this Journal*, 1912, xxx, p. 102.

¹⁸ PORTER and BEYER: *this Journal*, 1900, iv, p. 283.

the splanchnic nerves, restored the response to depressor stimulations quantitatively to the normal, thus confirming their results obtained by the simultaneous stimulation of the cut splanchnic and depressor nerves. In our opinion this might be interpreted in several different ways. It might mean that the splanchnic area plays only an insignificant rôle in the depressor fall — a conclusion which is contradicted by the plethysmographic results of Bayliss — or it might mean that the other vascular areas compensate automatically for the loss of the splanchnic area; or, finally, it might mean that the response of the vasomotor centres to depressor stimulation is heightened by the increased blood pressure, or possibly by the saline solution directly.

Our experiments were made on cats, with the splanchnic nerves intact, 20 c.c. per kg. of 0.9 per cent sodium chloride solution being injected intravenously.

The average of five experiments are as follows:

Before injection, depressor fall from 77 to 59 = 18 mm., or 23.3 per cent.

During injection, depressor fall from 95 to 73 = 22 mm., or 23.2 per cent.

After injection, depressor fall from 96 to 73 = 23 mm., or 23.9 per cent.

These show a greater absolute fall, the percentile fall being practically unaltered. These changes are no greater than what would correspond to the increased blood pressure. The perfusion flow showed a distinctly greater dilation (Experiments C 107 and 108). It must therefore be concluded that saline injection increases the excitability of the vasodilator centre by raising the level of blood pressure. However, it does not restore a centre which has been paralyzed; that is, it is ineffective in animals in which the depressor response had completely disappeared.

It should be pointed out that this stimulation of the vasodilator centre does not invalidate Porter and Beyer's use of normal saline injections in their special problem.

Hemorrhage. — A slight hemorrhage may somewhat increase the percentile response to depressor stimulation. Thus, in Experiment 103, the depressor response before bleeding was a fall of 7 per cent from the level of 100 mm. After a slight bleeding from the femoral artery the depressor response was a fall of 8.6 per cent from the level of 93 mm. As the hemorrhages increase, the fall is progressively diminished and finally abolished. The perfusion reaction is preserved longer. This may be seen from a tabulation of Experiment 108:

Blood pressure fall.		Perfusion flow.
Before hemorrhage	115-68 = 47 mm. = 41 per cent.	28-40 = 12 units.
At end of first hemorrhage .	56-46 = 10 mm. = 18 per cent.	29-38 = 9 units.
At end of second hemorrhage	50-40 = 10 mm. = 20 per cent.	28-32 = 4 units.
Before third hemorrhage . .	60-42 = 18 mm. = 30 per cent.	26-36 = 10 units.
At end of third hemorrhage .	36-36 = 0 mm.	32-32 = 0 units.
After reinjection of blood .	60-60 = 0 mm.	42-42 = 0 units.

The failure to recover by injection of the blood was also seen in the other experiments. In Experiment 103, for instance, the blood pressure was restored to the original level (100 mm.) by the reinjection of the blood, but the depressor remained ineffective.

It may therefore be concluded that the lesser response of the blood pressure with moderate hemorrhage is due to the lesser mass of blood, the centre retaining its activity, but when the pressure falls below 45 mm. the centre becomes paralyzed (in cats) usually without recovery.

Nitroglycerine.—The effects of nitroglycerin on the depressor reflex were investigated in three cats; 0.5 to 1 mg. per kg. of nitroglycerin being injected intravenously. The average figures for the blood pressure response on depressor stimulation are:

Before injection of nitroglycerin, 145-105 = 40 mm., or 27.6 per cent.
 At depth of nitroglycerin fall, 96-70 = 26 mm., or 27.1 per cent.
 Several minutes later, 105-78 = 27 mm., or 25.7 per cent.

It appears from this that the effects of depressor stimulation and nitroglycerin are strictly additive. The percentile response to depressor stimulation is not materially altered in the nitroglycerin fall, and the absolute response is practically the average response at the corresponding level, without nitroglycerin. (According to our curve depressor stimulation without nitroglycerin would lower the pressure from 96 to 73 mm.) The strictly additive results suggest that the mechanism of nitroglycerin dilation and that of depressor dilation are not identical.

CONCLUSIONS.

1. The dilator reaction of the vasomotor in response to vagus-depressor stimulation in the cat can be shown by the perfusion method. The pulse pressure (membrane manometer) is generally diminished in the depressor fall.

2. The depressor fall, both absolute and percentile, increases as a smooth curve with the level of blood pressure. The results are therefore different in cats and rabbits.

3. Occlusion of the aorta diminished the depressor fall; sometimes, however, a large fall may occur even during compression. Such falls must be due to a cardiac factor, since the response of the dilator centre is not increased by aortic compression. The peripheral vasomotor apparatus may be impaired for some time after aortic compression.

4. The vascular dilation from depressor stimulation affects the heart indirectly. Ordinarily the cardiac volume is diminished, but without altering the amplitude of the excursions. But when the depressor effect is excessive (as under high initial blood pressure), or when the cardiac efficiency is already low (as under low initial blood pressure), then the impaired blood supply weakens the heart.

5. Strychnin, in small doses, increases the depressor response, independently of its own effect on blood pressure.

Infusion of normal saline increases the depressor response, proportional to the rise of blood pressure.

Hemorrhage at first increases, then diminishes, the depressor response. The lessened response is at first due to the lesser volume of blood, but with severe hemorrhage the centre itself suffers.

During the nitroglycerin fall depressor stimulation produces a fall corresponding to the level of blood pressure. The effects of nitroglycerin and of depressor stimulation are therefore strictly additive, indicating that they involve a different mechanism. A similar simple summation is observed with depressor stimulation and traction of the carotid artery.

THE INFLUENCE OF ADRENALIN, MODIFIED BY SALTS, ON THE BLOOD PRESSURE IN THE CAT.

By I. R. BURKET.

[From the Laboratory of Physiology in the University of Kansas.]

METHOD.

THE usual procedure was employed in obtaining blood pressure records from the cat: the cannula was inserted into the carotid artery and connected to a mercury manometer; a saturated solution of sodium carbonate was used as a transmission fluid. In the first few experiments the test solutions were injected into the femoral vein with a hypodermic syringe, but because of the rapid clogging of the vessel from clotting as a result of this method, a glass cannula was tied into the vein and the injections made through this. The solutions were placed in the cannula and forced into the vein by compression of the attached rubber tube, after which the cannula was thoroughly washed to remove all traces of the substance used.

In preparing the salts for injection, volumetric solutions (molecular where the solubility permitted) of chemically pure substances were made, using doubly distilled water as a solvent. Before administration these were diluted to the desired concentration with doubly distilled water. The stock solution of adrenalin was made from Parke, Davis & Co.'s base — 1 mgm. to each cubic centimetre of water acidulated with 0.004 per cent hydrochloric acid — and was discarded when two weeks old. The dose given in each instance was 1 c.c. of the solution.

The effect of adrenalin on the cat. — When injected intravenously, adrenalin acts very quickly, a maximal rise in pressure taking place within a few seconds, succeeded by a return to normal in a few seconds more, the whole effect lasting on an average less than one minute. The after effect of fresh adrenalin is especially interesting: the curve does not stop when it has reached normal pressure after the rise, but

continues to fall till the manometer has shown a decrease equal to or greater than the original rise (Fig. 1). I have been unable to find any such action recorded in literature dealing with adrenalin.¹ Lohmann² showed that, in certain admixtures of adrenalin and cholin, both substances might act separately, the adrenalin causing a rise in pressure



FIGURE 1.—Four fifths the original size. Carotid pressure. Hg manometer. Adrenalin causes an after depression, sometimes greater than the original rise. Time, 2 seconds. The blood pressure at the beginning of the tracing is 160 mm. Hg; at the lowest point in the curve it is 130 mm. Hg.

followed by the cholin depression. To prove that such was not the case in these experiments the adrenalin was removed by oxidation; a 1:10,000 solution was made alkaline and allowed to stand for several days, when, upon delicate chemical tests, the adrenalin was found to be entirely absent; this solution after being neutralized produced upon injection neither a fall nor a rise in blood pressure, proving the absence of cholin as well as adrenalin.³

The effect of salts with adrenalin.⁴—The after effect of adrenalin caused me to look for a substance which, when injected simultaneously

¹ Since this article was prepared for publication a similar finding has been recorded by v. LEERSUM: *Archiv für die gesammte Physiologie*, 1911, cxlii, pp. 377-395.

² LOHMANN: *Archiv für die gesammte Physiologie*, 1907, cxviii, pp. 215-227.

³ I have recently been informed by E. R. WEIDLEIN that the methods at present in use for the purification of adrenalin are at fault, and that Parke, Davis & Co.'s crystalline adrenalin—considered 100 per cent pure by SCHULTZ (*Washington Hygienic Laboratory*, 1910, Bulletin 61, p. 25)—really contains an impurity which may account for the after depression. Mr. WEIDLEIN's paper will appear shortly.

⁴ Parke, Davis & Co.

with it, might prevent the undesirable depression without in any way diminishing the initial rise produced by adrenalin. This substance was found in barium chlorid. In even as great dilution as a $1/128$ *m* solution, barium chlorid injected alone will cause a marked stimulation of the heart action and a rise in blood pressure. When injected

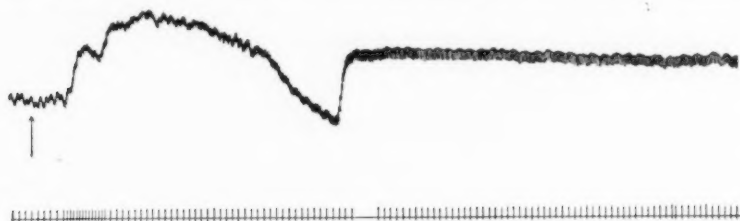


FIGURE 2.—Carotid pressure. Hg manometer. BaCl_2 sustains pressure and prevents after depression. The temporary fall occurred in a few cases in which the older stock adrenalin was used. Time, 2 seconds.

with adrenalin, the rise caused by the latter is noticeably augmented, the pressure does not fall so rapidly, and the after depression is entirely absent (Fig. 2). The strengthened heart beat may be observed for fifteen minutes or more after injection.

In order to determine whether the mere addition of a given amount of liquid (in these experiments 1 and 2 c.c.) to the circulation would in itself cause any change in pressure, several injections of Ringer's solution⁵ were made and were found to produce no effect. The same statement holds true concerning the effect of Ringer's solution on the change of pressure caused by adrenalin.

The next salt experimented with was sodium chlorid. Injection of an $m/8$ solution (which is of nearly physiological osmotic pressure) caused no change either in blood pressure or heart action, and an $m/2$ solution produced only a slight rise in blood pressure; these

⁵ NaCl , 0.7 per cent; CaCl_2 , 0.026 per cent; KCl , 0.03 per cent.

results corroborate the findings of Hyde,⁶ Mayor,⁷ and Ritter.⁸ Nevertheless, from this work, sodium chlorid in the doses given may be considered a neutral salt. But when injected ($m/2$) with adrenalin, it caused a decrease in the original rise; it did not, however, affect the length of action, nor did it prevent the after fall which usually follows the adrenalin rise.

Potassium chlorid in dilutions of $m/32$ may be considered practically neutral, both alone and in combination with adrenalin. Twice as concentrated solutions, however, have a depressing effect on blood pressure and heart rate when given alone (Fig. 3), and just the opposite effect in connection with adrenalin (Fig. 4). This latter result may be explained by the statement of Howell,⁹ that potassium chlorid increases

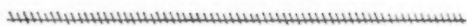


FIGURE 3. — Carotid pressure. Hg manometer. KCl causes a depression of blood pressure and heart action. Time, 2 seconds.

vagus irritability in certain doses, while in larger ones it depresses; the dose of potassium chlorid in connection with adrenalin was perhaps sufficient to depress the vagus, while alone it acted as a vagus stimulant, therefore slightly lowering the blood pressure.

Calcium chlorid and magnesium sulfate have a depressing effect on blood pressure, both when given alone and when administered with adrenalin. Meltzer and Auer¹⁰ obtained the same results from

⁶ HYDE: this Journal, 1908, xxiii, p. 201.

⁷ MAYOR: Journal de physiologie, 1902, iv, 3, p. 425.

⁸ RITTER: Deutsches Archiv für klinische Medizin, 1910, c, p. 11.

⁹ HOWELL: this Journal, 1906, xv, pp. 280-294.

¹⁰ MELTZER and AUER: this Journal, 1906, xv, p. 387.

the injection of magnesium, but they used much larger doses, causing complete inhibition of the respiration as well as a fall in blood pressure.

Some experiments were undertaken with the phosphates as the result of a test which showed the adrenalin base to contain traces of these substances. The phosphates used were the acid, neutral, and alkaline sodium salts. No definite effect could be observed, either

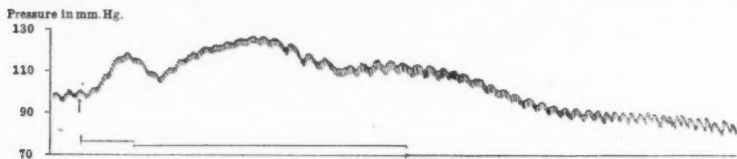


FIGURE 4. — Two thirds the original size. Carotid pressure. Hg manometer. KCl augments adrenalin pressure (see text).

when used alone or when given with adrenalin; the acid phosphate, however, increased the pressure somewhat, but had no effect on the adrenalin rise, while the neutral and alkaline salts were without effect when used alone, and only the alkaline one, Na_3PO_4 , caused any change when given with adrenalin; the change produced here was a marked depression, sometimes even complete neutralization, of the adrenalin; but this was probably due to oxidation of the adrenalin (which takes place readily in an alkaline solution, see above) rather than to any specific effect of the tri-sodium phosphate itself.

Another substance which, being a waste product in the body, and hence normally coming into contact with other constituents of the blood, would be of interest in this connection, is the purin derivative, uric acid. This compound alone causes a decided rise in mammalian blood pressure; its effect on heart action, however, is very insignificant, being in a majority of cases quite neutral, in others causing only a slight increase in heart rate. It exhibits a peculiarity when injected with adrenalin in that it causes a decrease in the normal adrenalin rise. Just why this seemingly paradoxical result should follow the combination of these two body products will probably not be understood until the action within the body of certain substances produced in normal metabolism upon the internal secretions, individually and in co-operation with each other, is better known.

Control injections of hydrochloric acid and sodium hydroxid of equal acidity and alkalinity to that of $m/16 \text{ NaH}_2\text{PO}_4$ and Na_3PO_4 ,

respectively, showed that the results obtained from these salts were not due to their reactions. The hydrochloric acid caused a slight depression, while the sodium hydrate had practically no effect upon the blood pressure. Acid solutions equal in strength to the acidity of 1:10,000 adrenalin had no effect whatever; an alkaline solution of equimolecular concentration was also neutral.

This work was done at the suggestion of Dr. Ida H. Hyde, for whose valuable aid I am much indebted.

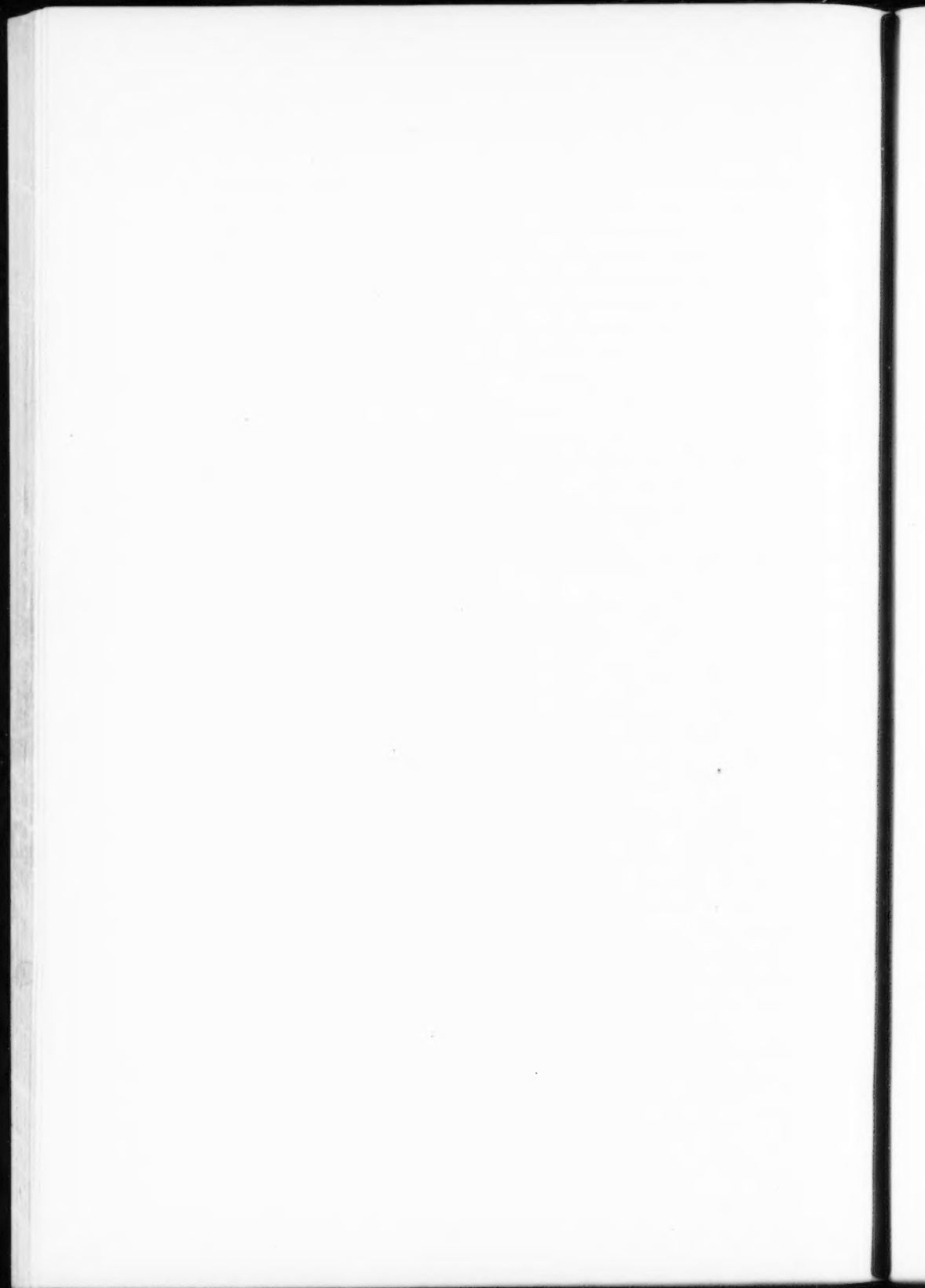
CONCLUSIONS.

1. Adrenalin has an undesirable after-depressing effect on mammalian (cat) blood pressure. This may be due to a hitherto unsuspected impurity, as suggested by Weidlein.³ The after effect may be counteracted by very small doses of barium chlorid. Barium chlorid also strengthens heart action and sustains high blood pressure for some time.

2. The waste product, uric acid, antagonizes adrenalin pressure, although alone it increases blood pressure.

3. The salts which cause a rise in pressure are: NaCl, $m/2$; BaCl₂, $m/128$ and $m/32$; uric acid; and NaH₂PO₄, $m/16$. Those lowering the pressure are: KCl, $m/16$ and $m/32$; CaCl₂, $m/16$; and MgSO₄, $m/8$. Those which show no definite action: Ringer's solution; NaCl, $m/8$; CaCl₂, $m/32$; and Na₂HPO₄, $m/16$.

4. Adrenalin pressure is augmented by: BaCl₂, $m/128$ and $m/32$; KCl, $m/16$. Salts which depress the effect of adrenalin are: NaCl, $m/2$; CaCl₂, $m/16$; MgSO₄, $m/8$; and uric acid.



THE ACTION OF ETHYL ALCOHOL AND WATER ON MUSCLE.

BY FREDERIC S. LEE AND M. LEVINE.

[From the Department of Physiology of Columbia University, New York.]

IN 1902 the senior author and Salant¹ published the results of a study of the action of ethyl alcohol on the skeletal muscles of the frog. They concluded that while in small quantity the alcohol was inactive, in medium quantity it exerted on the muscle a favorable action, which was characterized by a quickening of both contraction and relaxation, the power of making a larger number of contractions and of performing a larger amount of work in a given time, a delay of fatigue, and the power of making a larger number of contractions and of doing more work before exhaustion sets in. When in large quantity the drug acted unfavorably, the phenomena being in general the reverse of the former. Four years later Hough and Ham,² using a similar method, confirmed in all essential points the favorable action of medium quantities of alcohol, but reported that qualitatively similar and even greater results were obtained by the injection of water or Ringer's fluid. Only eleven experiments were performed with water alone and only five with Ringer's fluid alone, yet the authors felt themselves justified in maintaining that the improvement in the working power of the alcoholized muscle was due not to the alcohol, but "to the increase of the circulating medium, or to the reduction of osmotic tension in the blood plasma, or to both of these causes combined." This claim, supported by apparently trustworthy if not very abundant, experimental evidence, seemed to necessitate a re-examination of the experimental basis of the conclusions of Lee and Salant. This the present authors have now made.

¹ LEE and SALANT: this Journal, 1902, viii, p. 61.

² HOUGH and HAM: Biological studies by the students of William Thompson Sedgwick, Boston, 1906, p. 321.

All of our experiments were performed, as before, on the gastrocnemius muscles of the common leopard frog, *Rana virescens*, during the months from February to June, on frogs that had been wintered in the laboratory. One hundred and fifty frogs were used. Curare was not employed. The muscles were excised and stimulated by a series of maximal break induction shocks at the rate of from fifty to sixty times in the minute. In each case the two gastrocnemii of opposite sides were employed in succession, one serving as a control. Their working powers were compared by measuring the total height, as recorded by the work adder, to which each muscle was capable of lifting the weight before exhaustion ensued. The weight was the same for the two muscles of each pair. After each contraction the lever returned to the original abscissa. Since the exact determination of the total amount of work performed in each case would have required for each experiment an elaborate calculation, owing to the peculiar construction of the work adder, and since we were seeking, not total amounts, but relative amounts of work, we employed the total height lifted as our criterion of efficiency.

Whoever has made such comparative experiments is aware of the fact that great individual differences are exhibited by similar muscles of opposite sides. We made forty comparisons of the right and the left normal excised gastrocnemii and found that in twenty cases the right, and in twenty cases the left, muscle performed the greater amount of work. While single muscles deviated greatly from the norm in the heights to which they were capable of lifting the weight, and while it often happened that several right muscles in succession surpassed the lefts, or *vice versa*, the percentage of total height to which the weight was lifted by the two muscles was 49 by the right and 50 by the left muscle, an approximate equality. These figures show that while in the long run equality of efficiency may be expected as between right and left muscles, it is not safe to draw comparative conclusions from the results of testing a small number.

For this reason we performed no less than forty experiments each on the action of ethyl alcohol and of water respectively. We have not used Ringer's fluid. The procedure was as follows: In each case, after the frog had been weighed, the right or the left thigh was ligated so as to occlude the blood flow. The ligated leg was then amputated and laid aside. Into the dorsal lymph sac of the frog was injected either

a 10 per cent aqueous solution of Squibb's absolute ethyl alcohol or distilled water, in the proportion of 0.16 c.c. of the liquid for each gram of body weight. While the alcohol or water was being absorbed the normal gastrocnemius of the amputated leg was prepared and attached to the work adder, and the record of its contractions up to exhaustion was made. Exhaustion ensued in from nine to seventeen minutes. When the absorption had proceeded for a time varying from twenty-five to sixty-seven minutes the frog was killed, and the record of the contractions of its remaining gastrocnemius was made. Right and left muscles were used for injection in alternate frogs. The results of the injection of the alcohol are given in Table I.

From this table it is obvious that there are widely diverging results in successive experiments, a fact that has been observed by many investigators of the action of alcohol. Yet in twenty-six, or 65 per cent, of the forty experiments the muscle that was under the influence of the drug lifted the weight to a greater height than the control muscle. The total height to which the weight was lifted by the forty alcoholized muscles exceeded that lifted by the forty normal muscles by 1379 mm., an average of 34 mm. for each muscle. This amounted for each muscle to a percentage increase of 8.35. The favorable action of the mixture of alcohol and water is here sufficiently obvious. This is most obvious when the mixture had acted for a period ranging from thirty to forty minutes, and subsequently passes away.

Is this augmentation of activity due to the alcohol itself? This Hough and Ham deny and they claim a similar beneficial action from distilled water. The results of our experiments with distilled water are given in Table II.

Here too there is great variation, but of the forty muscles thus treated with water only fourteen, or 35 per cent, showed an augmented effect, while the larger remainder were unable to lift the weight to the normal height. The sum total of this decreased lift amounted to 786 mm., or 19 mm. for each of the muscles, which is 0.95 per cent. Thus mere "increase of the circulating medium," or "reduction of osmotic tension," or whatever change the water was responsible for, far from acting favorably upon the working power of the muscle, on the whole slightly diminished it.

We can therefore see no escape from the conclusion that in our experiments with dilute ethyl alcohol the observed augmentation of

TABLE I.

Normal muscle. Total height of lift in mm.	Muscle after alcohol.		Difference in height of lift. + = alcohol; - = normal.	
	Duration of action of alco- hol in minutes.	Total height of lift in mm.	Total.	Percentage.
666	30	814	+148	+22.2
609	31	747	+138	+22.6
784	27	741	-43	-5.4
792	67	579	-13	-1.6
557	39	739	+182	+32.6
507	38	637	+137	+27.0
591	45	602	+11	+1.8
377	39	532	+155	+41.1
555	36	605	+50	+9.0
717	43	651	-66	-9.2
807	38	719	-88	-10.9
654	38	710	+56	+8.5
304	30	344	+40	+13.1
447	41	596	+149	+33.3
721	47	765	+44	+6.1
712	40	663	-49	-6.8
535	39	556	+21	+3.9
561	37	706	+145	+25.8
511	39	548	+37	+7.2
424	39	381	-43	-10.1
954	37	840	-114	-11.9
825	36	900	+75	+9.0
789	55	590	-199	-25.2
780	53	703	-77	-9.8
525	40	610	+85	+16.1
526	39	518	-8	-1.5
411	40	500	+89	+21.6
748	40	750	+2	+2
710	39	670	-40	-5.6
842	37	783	-59	-7.0
638	40	702	+64	+10.0
544	38	547	+3	+5
235	38	386	+151	+64.2
457	40	613	+156	+34.1
522	40	445	-77	-14.7
663	33	514	-149	-22.4
398	37	417	+19	+4.7
583	38	666	+83	+14.2
665	37	799	+134	+20.1
788	38	1008	+220	+27.9

TABLE II.

Normal muscle. Total height of lift in mm.	Muscle after distilled water.		Difference in height of lift. + = water; - = normal.	
	Duration of action of water in minutes.	Total height of lift in mm.	Total.	Percentage.
627	30	687	+60	+9.5
678	50	703	+25	+3.6
564	47	447	-117	-20.7
667	43	623	-44	-6.5
635	40	671	+26	+4.0
448	42	395	-53	-11.8
413	35	503	+90	+21.7
811	45	710	-101	-12.4
856	43	704	-152	-17.7
646	40	554	-92	-14.2
435	40	522	+87	+20.0
585	43	799	+214	+36.5
580	35	662	+82	+14.1
975	47	707	-268	-27.4
587	40	998	+411	+70.0
633	38	413	-220	-34.7
563	35	533	-30	-5.3
302	34	282	-20	-6.6
648	34	621	-27	-4.1
802	32	720	-82	-10.2
733	32	552	-181	-24.6
480	35	600	+120	+25.0
296	31	306	+10	+3.3
523	34	455	-68	-13.0
660	34	516	-144	-21.8
770	32	542	-228	-29.6
426	32	424	-2	-.4
458	37	247	-211	-46.0
744	34	796	+52	+6.9
639	36	707	+68	+10.6
784	35	737	-47	-5.9
228	35	375	+147	+64.4
540	37	517	-23	-4.2
538	37	525	-13	-2.4
624	43	604	-20	-3.2
735	45	697	-38	-5.1
564	45	472	-92	-16.3
617	34	594	-23	-3.7
582	40	560	-22	-3.7
584	44	724	+140	+23.9

working power was due to a specific action of the alcohol on the muscle itself. Lee and Salant's original deduction thus seems to have been justified. This augmentation of working power is temporary and passes over ultimately into the opposite condition in which less work can be done.

A similar preliminary and transient augmentation seems to have been observed by most investigators of the action of alcohol on the human being, although it does not appear to be found with all subjects. Some have not found it at all and have even denied its existence.³ In Ioteyko's⁴ experiments it was pronounced in two out of seven cases tested by means of the ergograph. We suspect that a study of a considerable number of individuals by a variety of methods and a careful analysis of the results — and most of the investigations heretofore performed have been lacking in these factors — would reveal that an augmentation of various physiological activities by alcohol is more common than is granted by some of the extremists. It may perhaps be found that such effects are often very fleeting and easily overlooked. Even where they have been observed, they are usually ascribed not to excitation of tissues, but to inhibition of inhibitory mechanisms or, where mental factors are involved, to suggestion. These explanations are largely hypothetical. Whether or not they will be proven to be correct in the case of the complex human body, they cannot be applied to the augmented working power of an excised muscle, either in such experiments as ours, or, especially in those of Lee and Salant, where the same effect was observed in curarized muscles. In the excised muscle we must assume the presence of physiological excitation, whatever the mechanism of such excitation may be.

Our conclusion is that ethyl alcohol when administered to frogs in moderate quantities is capable of augmenting the working power of the skeletal muscles.

³ R. FOERSTER has recently summarized the literature on the relation between alcohol and muscle work in PFLÜGER'S *Archiv für die gesammte Physiologie*, 1912, Bd. 144, p. 51. For a fuller and critical discussion see RIVERS: *The influence of alcohol and other drugs on fatigue*; The Croonian Lectures, 1906, London, 1908. This author is strongly wedded to the explanation of augmentation by suggestion.

⁴ J. IOTAYKO: *Les lois de l'ergographie*, Brussels, 1904. Also published in *Bulletins de l'Académie royale de Belgique (Classe des sciences)*, No. 5, May, 1904, p. 557.

OBSERVATIONS ON THE PHYSIOLOGY OF PURKINJE TISSUE.¹

By JOSEPH ERLANGER.

[From the Physiological Laboratory of Washington University, St. Louis.]

INTRODUCTION.

THE structures now generally known as Purkinje fibres were first seen and described by Purkinje in 1845.² Since then the Purkinje fibres have frequently been the subject of histological investigation, the results of which have formed the basis of inferences as to their functional activities.³ Only, however, after their true anatomical relations to the conducting system of the heart had been brought to light in 1906 by the painstaking studies of Tawara,⁴ did it become possible to form any rational idea as to the real significance of the Purkinje fibres. Tawara showed that the auriculo-ventricular bundle is composed of them, and, in addition, that the bundle does not terminate, as had been supposed, at the point where it enters the muscular septum of the ventricles, but continues as a closed system to the most distant parts of the ventricles. The path of the Purkinje fibres, as described by Tawara, is as follows: On the upper edge of the muscular septum of the ventricles the bundle of His divides into two branches, a right and a left, which continue down the respective sides of the septum subendocardially. The left limb, with which alone the present problem concerns itself, goes mainly to the anterior and posterior

¹ Reported before the American Physiological Society in December, 1911. See Proceedings of American Physiological Society, this Journal, 1912, xxix, p. xxv.

² PURKINJE: MÜLLER'S Archiv für Anatomie. Quoted by RANVIER: *Traité technique d'histologie*, Paris, 1889.

³ See HEINZ: *Handbuch der experimentellen Pathologie und Pharmacologie*, 1905, i, p. 652, Jena; and NICOLAI in NAGEL'S *Handbuch der Physiologie des Menschen*, Braunschweig, 1905, i, p. 810.

⁴ ZEIGLER'S *Beiträge*, 1906, xxxix, p. 563.

papillary muscles, arborizations extending from the latter over the whole inner surface of the left ventricle. A part of the path to the papillary muscles lies through certain of the false tendons bridging the cavity of the ventricles. It should not be forgotten in this connection that the conducting system of the heart contains as possibly active tissue, not alone Purkinje fibres, but also nerve elements, with which indeed, according to Wilson,⁵ Engel,⁶ and others, the bundle of His and all of its ramifications seem to be rather richly supplied. Since in the present state of our knowledge it is impossible to dissociate the functions of these two tissue elements, it is preferred to regard the ventricular portion of the conducting system as composed of *Purkinje tissue*, a term which will be used in this paper to include not alone Purkinje fibres, but also the nerve elements everywhere associated with them.

Tawara was led by his anatomical studies to draw certain inferences as to the physiology of the conducting system. He expresses it as his opinion⁷ that, "contrary to the physiologists, it is necessary to assume a rapid rate of conduction in the fibres of the auriculo-ventricular bundle. An arrangement which carries the impulses in closed paths directly to the most distant wall . . . is intended to permit the impulses to act upon all points of the ventricular wall as nearly simultaneously as possible. To accomplish this, however, the impulse must travel faster in the uniting bundle than in the rest of the ventricular musculature." He, however, admits "the possibility of a certain delay in the transmission of the impulse in the so-called node."

The present experiments represent an attempt to acquire some specific knowledge of the physiology of Purkinje tissue, and incidentally to put to the test of experiment the inferences from anatomy as to the functions of the auriculo-ventricular bundle.

In approaching the problem the first consideration was to find Purkinje tissue in some structure that would readily lend itself to physiological experimentation. The close association of the bundle of His with ventricular musculature seemed to exclude the former. Since the present research was begun, however, Hering has published some experiments in which the bundle of His was used for this very

⁵ WILSON: Anatomical record, 1909, viii, p. 262.

⁶ ZEIGLER's Beiträge, 1910, xlviii, p. 499.

⁷ TAWARA: Das Reizleitungssystem des Säugetierherzens, Jena, 1906, p. 186.

purpose.⁸ Hering claims it is possible, in the dog, to so stimulate, both electrically and mechanically, the cut surfaces of the auriculo-ventricular bundle that the stimulus does not escape to the surrounding structures. Pending confirmation of this claim it would seem best to accept with caution Hering's conclusion, arrived at by the aid of this method, to the effect that Purkinje tissue is independently irritable, and that possibly the rate of conduction in Purkinje tissue is approximately the same as in heart tissue. The latter conclusion, it should be added, rests upon the further assumption that the peripheral junction of the Purkinje elements with the ventricular musculature lies some distance removed from the point of application of the stimulus.

The primary method employed in the present research was suggested by the statement of Retzer that in a "large number of pigs' hearts the moderator band consists of nothing but fibres of the conducting system, together with the constantly accompanying nerves and blood vessels."⁹ Here apparently, it was thought, is a structure that would lend itself well to physiological experimentation. It was deemed advisable, though, before performing any experiments on the moderator band, to assure ourselves of the correctness of Retzer's observation. This task was assigned to M. S. Petersen in 1909.¹⁰ Petersen, finding that the moderator band of the pig's heart consists chiefly of heart muscle, extended the problem so as to include a study of the interventricular bands of other animals. He found that the moderator band of the sheep's heart also contained heart muscle, but that, in confirmation of "Miss DeWitt's observations . . . some of the interventricular bands of the beef's heart consist of Purkinje fibres and connective tissue, exclusively." Petersen consequently concludes that it should be possible to obtain definite results with the interventricular bands of the left ventricle of the beef's heart "by following the physiological studies with a histological examination." This precaution, it should be added, has been consistently followed during the course of the present research.

The beef's heart has another advantage over other hearts in this

⁸ HERING: *Archiv für die gesammte Physiologie*, 1910, CXXXI, p. 572.

⁹ RETZER: *Johns Hopkins Hospital Bulletin*, 1909, xx, p. 168.

¹⁰ The work was completed in May, 1910, when it was filed with the Librarian of the University of Wisconsin as a thesis for the degree of Master of Science.

connection in that its conducting system has been studied with the greatest of care. Miss DeWitt's wax model of this heart¹¹ represents the most complete reconstruction we possess of the conducting system of any heart. Although there is great variability as to detail, the false tendons of the beef's heart are usually arranged as follows: One false tendon each arises from the anterior and posterior subdivisions of the left branch of the bundle of His and passes directly to the anterior and posterior papillary muscles, respectively. These main false tendons we shall designate anterior and posterior. During their course across the cavity of the ventricle, the tendons may branch, the branches either continuing in the same general direction as the main tendon, or passing back to fuse with the septum. In our experiments we have made use mainly of the anterior and posterior false tendons of the left limb of the bundle of His and their branches.

METHODS.

I. *Treatment of excised false tendons with salt solutions, etc.* — At first the attempt was made to determine whether the false tendons of the left ventricle of the bullock's heart would beat when placed under the conditions that permit mammalian auricular strips to develop spontaneous rhythmicity.¹² The false tendon, excised from the recently killed animal, was treated as follows: (a) It was immersed for some time in Locke's solution at a temperature of 35° C., and then, from time to time, temporarily removed from the bath and stimulated with the interrupted induced current. (b) The strips were then placed for a few moments in a bath consisting of a 1 per cent solution of potassium chloride at a temperature of 35° C. After reimmersion in the bath of Locke's solution the strip was again treated as under (a). These steps were taken in the hope that they would counteract any such inactivity as might possibly have been due to fibrillation. (c) The false tendons were also transferred to a bath of sodium citrate, $\frac{1}{8}$ m at 35° C., and returned to the bath of Locke's solution, receiving in each bath the treatment described under (a).

No results whatever were obtained in four such experiments. Contractions were never observed. Histological examination showed that

¹¹ DEWITT: Anatomical record, 1909, iii, p. 475.

¹² ERLANGER: this Journal, 1910, xxvii, p. 87.

three of the four strips studied contained Purkinje tissue only, while one contained some heart muscle. Evidently the method suffices to impart irritability or rhythmicity neither to beef's ventricular heart muscle nor to Purkinje tissue.

II. Perfusion of false tendon. — It became obvious, as a result of the failure of Method I, that in order to obtain results it would be necessary to perfuse the false tendons. In the hope of being able to avoid the necessity of perfusing the whole heart, an undertaking fraught with some difficulty, direct perfusion of the false tendons alone was first attempted. When the septum of the ventricles is cut across parallel to and about 1 cm. below auriculo-ventricular junction, blood vessels can be seen in the left limb of the bundle of His, coursing apparently toward the main false tendons arising from it. These arteries, like the false tendons themselves, vary greatly in size and arrangement. Very often, however, one can be found that is large enough to take a very small cannula.

The preparation is then made as follows: The septum and false tendons are exposed and the distal ends of the latter are cut. A block of septum, from which the main false tendons arise, is excised, the uppermost cut being in the position described above. The cannula is inserted into the artery and the strip is perfused with Locke's solution in the usual way.

Although a great many attempts were made to perfuse false tendons in this way, a satisfactory flow was obtained only three times. In none of the experiments did the false tendons, or any other part of the strip, contract, even after repeated temporary perfusion with 1 per cent potassium chloride. False tendons from the three cases of successful perfusion were studied histologically. They all contained bundles of Purkinje tissue, while one contained heart muscle in addition, and this in considerable amounts. Evidently this method, like the previous one, cannot be counted upon to yield results.

III. Perfusion of the whole heart. — No other course remained open then than to perfuse the whole bullock's heart. Inasmuch as an experiment so complex as the perfusion of the beef's heart could be satisfactorily handled only in a laboratory provided with all the facilities for physiological experimentation, it became necessary to transfer the place of work from the slaughter house, where all of the above-described experiments were made, to the University laboratory. It

is not necessary to describe the perfusion apparatus employed further than to say that it was built so as to keep in continuous circulation about 10 litres of solution. At first hearts were brought to the laboratory from the slaughter house. With these, however, no success was attained. When received, they were all firmly contracted and could not by any means whatever be made to beat.

Then hearts were obtained from small calves slaughtered in the laboratory. In spite of this facility the greatest difficulty was experienced in obtaining and maintaining contractions, a difficulty which others before us seem to have had.¹³ The trouble consisted in the liability of the ventricles of the calf's heart to fibrillate. The calf's ventricles certainly fibrillate upon much less provocation than any other heart (rabbit's, cat's, dog's) the author has had occasion to perfuse. After beginning the perfusion, the first or second auricular impulse that succeeds in reaching the ventricles often starts them fibrillating, and the very weakest electrical stimulus that produces any reaction at all usually has the same effect. Mechanical stimulation seems to be equally deleterious. Furthermore, it is not often that fibrillation of the calf's heart can be stopped, as in other hearts, by means of temporary perfusion with potassium chloride solution, and not infrequently when potassium chloride had stopped fibrillation, the heart, upon reperfusion with Locke's solution, either has not contracted at all and has lost its irritability completely, or has remained irritable, although not spontaneously rhythmical, or, as in a very few cases, has been spontaneous for only a very short time. After the first few moments of perfusion the auricles, with two exceptions, have ceased entirely to contract. This tendency of the calf's heart to fibrillate and then to completely lose its irritability may not be due wholly to a peculiar sensitiveness of this heart to stimulation; it is possible that the Locke's solution ordinarily employed in perfusion experiments is not suited to the calf's heart. As a matter of fact, it was found, after many combinations had been tried, that the heart did best when fed with a mixture of equal parts of calf's blood and a 0.9 per cent sodium chloride solution.

In the first experiments on the calf's heart the false tendons were exposed by cutting a large opening into the outer wall of the left

¹³ MAGNUS-ALSLEBEN: *Archiv für experimentelle Pathologie und Pharmakologie*, 1911, lxiv, p. 228.

ventricle between the two papillary muscles. The cut blood vessels were tied by transfixion. Four experiments of this kind were performed; owing, however, to the difficulties mentioned above, only one yielded any results at all. In this case the heart had lost its spontaneity, although it still responded, and for many hours, to electrical stimulation.

Then, in order to avoid the danger of throwing the ventricle into fibrillation through unnecessary manipulation, the false tendons were exposed by cutting away the left auricle and the mitral valves. The false tendons thus exposed were not quite so accessible as in the earlier experiments. Still, in hearts with conveniently placed tendons and by the employment of suitable appliances (including aurists' forceps and scissors, platinum electrodes on bent handle and a small electric light which could be lowered in the cavity of the ventricle so as to obtain good illumination), the false tendons could be manipulated very satisfactorily. In these experiments it became necessary to prevent the accumulation in the ventricle of the fluid which oozed from the smaller cut vessels and which escaped through the aortic valves which invariably leaked to a certain extent, even under the low pressure of 60 mm. of mercury that was maintained in most of the experiments. At first the drainage was accomplished by means of aspiration; later the blood was drained off by means of a small cannula thrust through the tip of the ventricle. The temperature of the perfusion fluid lay between 31° and 36° C. In any one experiment it usually remained constant within 1° C. Records were made of the moment of stimulation by means of a signal, of the movements of the outer wall of the heart by means of tambours, and of the time in fifths of seconds upon a Hürthle kymograph moving at the rate of over 60 mm. per second.

OBSERVATIONS.

Only eleven of the twenty-seven perfusion experiments have yielded results, while only six can be considered entirely satisfactory from a technical standpoint. Nevertheless, the opportunity was afforded by these successful experiments, in which the heart usually continued to beat for many hours, to try the same procedure over and over again. This fact is of some importance, since by frequent repetition alone is

it possible to reduce to a minimum the disturbing effects of the many variable and largely uncontrollable factors that one has to deal with when results are obtained by stimulating a spontaneously rhythmical tissue whose irritability varies throughout each cycle. Other steps taken to minimize these factors will be mentioned from time to time below.

Histological findings. — It may be stated here once and for all, that all of the false tendons of the successful experiments of this series contained Purkinje tissue. Two of them contained heart muscle in addition; one (Exp. 10) in considerable amount, the other (Exp. 19) a single small bundle. The presence or absence of heart muscle had no obvious influence upon the behavior of the false tendons.

Independent irritability of false tendons. — The experiments have demonstrated that false tendons containing as their active constituent only Purkinje tissue are irritable in that when they are stimulated electrically the ventricles respond with contractions. There can be no question in these experiments of an escape of the current to the ventricular muscle. In some of the trials the electrodes (both bipolar and unipolar methods of stimulation have been employed) were applied to the false tendons a centimetre or more from their insertion into the ventricular wall; while the strength of the stimulus eliciting a reply from a false tendon in many cases has not sufficed to elicit a reply when applied directly to the ventricular wall.

Relative irritability of Purkinje and heart tissue. — This brings up the question of the relative irritability of Purkinje and heart tissue. First, however, it is necessary to call attention to the relatively unfavorable conditions to which the false tendons are exposed in these experiments. Being narrow strands of exposed tissue, the false tendons must feel the effects of cooling and drying much more quickly than the ventricular walls. The difference is minimized, however, by the fact that the ventricular cavity, in which the false tendons hang, acts practically as a moist and warm chamber. The additional precaution was, however, taken of permitting the ventricles from time to time to fill with the perfusion fluid. The stimuli were break shocks supplied by a vertical induction coil with 5000 windings in the secondary; the primary current was supplied by two Edison-Lelande elements. In comparable tests the shocks were applied at as nearly as possible the same rate and, in those cases in which the ventricles were beating sponta-

neously, as nearly as possible in the same phase of each cycle. It is obvious that it was impossible to meet satisfactorily all the requirements of an absolutely quantitative experiment; but, as has been said above, the difficulties are minimized by the large number of readings made. Two sets of figures, each of which represents the average of a number (usually 4 to 10) of readings, will serve to indicate the relative irritability of the tissues, one set (Table I) indicating qualitatively

TABLE I.
EXPERIMENT 11.

Position of coil.	Part stimulated.	Results.
16.5	Septal end of false tendon	Responds to all breaks.
14.5	Septum, some distance from white streak	Responds to most breaks.
14.0	Septal end of false tendon	Responds to most breaks.
14.0	Septum, as above	No response.
14.0	Septal end of false tendon	At first no response, later to breaks.
14.0	Septum, as above	No response.
14.0	Septal end of false tendon	Responds occasionally.

that the threshold of the septum is higher than that of the false tendons; the other set (Table II) giving quantitative results.

The figures show that the irritability of the false tendons, of the inner and of the outer heart wall, to induction shocks, while possibly decreasing slightly in the order given, is almost alike; whereas the irritability of the white streak, that is, the left branch of the auriculo-ventricular bundle, as determined by applying electrodes to the endocardium immediately over it, is perceptibly lower than that of the parts of the heart mentioned above. No explanation of this last observation is attempted.

The effect of the strength of stimulation. — The effect on the reaction time of the ventricles of varying the strength of the stimulus applied to a false tendon has not been studied for the purpose of obtaining exactly quantitative results. From the records it is clear, however, that the reaction time is slightly shorter with stronger than with weaker stimuli. Thus in one experiment (No. 11) the reaction time to make

and break shocks with the coil in the same position was 0.1 and 0.08 second respectively. While in another experiment (No. 20) with the secondary coil at 26 cm. (threshold), 25.5 and 25.0 cm., the reaction times were 0.117-, 0.11+, and 0.098 second, respectively.

Irritability of true tendons. — To test the irritability of true tendons, the tendon was cut close to its insertion into the valve leaflet, and

TABLE II.

EXPERIMENT 20.

Threshold position of coil.	Part stimulated.	Remarks.
19.5	Right ventricle	In rapid succession.
19.5	Septal end of false tendon	
19.0	Outer wall of right auricle	
20.0	Septal end of false tendon	In rapid succession.
19.0	Outer wall of right ventricle	
20.0	Septal end of false tendon	
19.5	Septum to right of white streak	In rapid succession.
20.5	Septal end of false tendon	
20.5	Septum to right of white streak	
22.0	Septum to right of white streak	In rapid succession.
19.0	Septum on white streak	
22.0	Septal end of false tendon	

stimulated close to its origin from the papillary muscle while recording the movements of the right ventricle. No responses whatever have been obtained even with strengths of stimulation far in excess of those that were efficient for false tendons and ventricular walls. Under the conditions of our experiments, therefore, the conclusion is justifiable that false tendons are neither irritable nor do they conduct.

Contractility. — Owing to the difficulty of reaching the false tendons and to the fact that they move with the part of the heart to which they remain attached, it was found impossible to record satisfactorily their movements. Throughout the research, however, the false tendons have

been closely observed for the purpose of determining whether they contract. The cut tendon lying against the smooth, moist endocardium, or hanging freely into the cavity of the ventricle would certainly have moved perceptibly had any of its elements contracted. In only one case did the false tendons seem to move independently of the heart wall. In this case the false tendons contained no heart muscle.

Rate of conduction in false tendons. — The rate of conduction in false tendons could be calculated if we knew either (1) the reaction time of the ventricles to stimulation of a cut false tendon at two points along its course and the distance between these points; or (2) the reaction time of the ventricles after stimulation of a cut end of a false tendon and of the ventricular wall near the insertion of that false tendon (granting the identity of the latent periods of false tendon and of ventricular muscle) and the length of the tendon. In discussing the results that have been obtained, it is particularly necessary to bear in mind that the criterion of response to stimulation is a contraction of the ventricles and that the latent period of the ventricles is affected by a number of different conditions. We mention, for example, (a) the phase of the cycle in which the extra stimulus is placed; (b) the rate at which the heart is beating; (c) the rate at which the repeated stimuli are applied; (d) the strength of the stimulus, etc. By using threshold stimuli, by placing them as nearly as possible in the same phase of the cardiac cycle, and by repeating them as nearly as possible at the same rate, these factors may be reduced to a minimum. Since, however, it is practically impossible to control all factors, it is to be expected that the figures representing the latent periods will vary somewhat amongst themselves.

Method 1. — Some of the typical results obtained by the first method mentioned above are given in Tables III and IV.

The difference in time in Experiment 15 averages approximately 0.011 second. The distance subtended by the electrodes was about 8 mm.¹⁴ The rate of transmission is, therefore, about 73 cm. per second. The average difference in time in Experiment 17 is 0.0135. The distance between electrodes was about 10 mm. The rate of antidromic conduction is, therefore, about 74.1 cm. per second.

¹⁴ The measurements of length were made on the cut flaccid tendon. They are probably much smaller than the distances between the same points in the uncut tendon.

Technical difficulties have stood in the way of determining the conduction time in the normal direction. Since, however, the reaction

TABLE III.
EXPERIMENT 15.

Threshold strength of stimulus.	Stimulation of septal stump of posterior false tendon.	Average latency in seconds.	Remarks.
20.0	Near end	0.06	In rapid succession.
20.0	Near base	0.06+	
16.75	Near end	0.08	In rapid succession.
16.75	Near base	0.057	
15.0	Near end	0.09	In rapid succession.
15.0	Near base	0.08+	

times to stimulation of the central and peripheral ends of a cut tendon are practically identical, it may be assumed that conduction time is approximately the same in both directions. In any event there can

TABLE IV.
EXPERIMENT 17.

Threshold strength of stimulus.	Stimulation of septal stump of anterior false tendon.	Average latency in seconds.
14.0	Near end	0.1025
14.0	Near base	0.089

be no reason for assuming that conduction time is slower in the normal than in the antidromic direction.

Method 2.—As has been said above, it might be supposed that Method 2 could be employed for the purpose of estimating the rate of conduction in false tendons. As a matter of fact the results that have been obtained with it, as shown by Table V, given below, are so variable as to indicate that factors are concerned which tend to obscure

that of conduction. For instance, the latent period following stimulation of the septum often is larger than that following stimulation of a false tendon. Just what the disturbing factors are has not been determined. It is not difficult, however, to mention some of the possibilities in the case. There might, for example, be a difference in the

TABLE V.

Experiment.	Latency of ventricular contraction with stimulation of false tendon.	Latency of ventricular contraction with stimulation of inner heart wall.
Experiment 17	0.133+	0.133-
	0.11+	0.112
	0.102+	0.101
Experiment 20	0.123	0.096+
	0.112	0.10
		0.1
Experiment 22	0.082	0.104
	0.084	0.11

latent period of response of heart muscle to the impulse delivered into it from Purkinje tissue on the one hand, and from electrodes on the other; or the location in which the impulses started in these two ways are delivered to the heart muscle might not be the same; or the relation of the muscle bands that first contract under the two methods of stimulation to the point on the ventricles connected with the receiving tambours might vary, etc., etc.

Hering found in the dog that the reaction time was the same (*Derselbe Grossenordnung*) with stimulation of the ventricular end of the cut auriculo-ventricular bundle and of the ventricles directly.¹⁵ The results given above in a general way confirm Hering, and at the same time direct attention to the inadequacy of the method. The bearing of these results on the question of the location of the union of Purkinje with heart tissue is discussed below.

¹⁵ HERING: *Archiv für die gesammte Physiologie*, 1910, cxxxi, p. 572.

Discussion of results bearing on conduction.—At the outset it should be recalled that the impulses that start contractions in these experiments must be interpolated between the spontaneous contractions. There is every reason for believing, therefore, that the induced impulse travels more slowly than the impulse started by spontaneous contractions, and that the faster rates observed, rather than the average rates, more nearly approach the normal. In any event it is justifiable to conclude that the rate of conduction in false tendons is at least 75 cm. per second. How this compares with the rate of conduction in the heart wall it is impossible to say. What is supposed to be the rate of conduction in heart muscle has been measured by a number of investigators by means of the action current. Owing to the fact, however, that the ventricles are not a parallel fibred muscle, and that it is not known where the impulse is delivered to this muscle, these observations have relatively little value. Indeed, the recent experiments of Clement¹⁶ indicate that all parts of the ventricular wall become negative at the same moment. For this reason it has not been deemed necessary to determine for comparison with our results the rate of conduction in the perfused calf's ventricle. It is not entirely without interest, however, to compare the rate of conduction in false tendons with the so-called rate of conduction in ventricular musculature. So far as we are aware the only figure that has been obtained from a study of the perfused heart is that of Schleuter. He found the rate of conduction in the dog to be 200 to 400 cm. per second.¹⁷ These figures, though, are of but little significance in the present state of our knowledge. The remarkable fact is that the rate of conduction in the long slender false tendons is as rapid as we have found it to be.

Direction of conduction in false tendons.—False tendons conduct in both directions and apparently with equal facility. In not a single experiment in which the heart and the false tendons were in a condition to respond did stimulation of either end of a cut false tendon fail to elicit a contraction of the ventricles. Neither was there any constant difference in the threshold value of stimulation in favor of one or the other direction. Perhaps of even greater interest is the fact that the latent period of stimulation, as has been mentioned

¹⁶ CLEMENT: *Zeitschrift für Biologie*, 1912, lviii, p. 110.

¹⁷ SCHLEUTER: *Archiv für die gesammte Physiologie*, 1902, lxxxix, p. 87.

above in another connection, is approximately the same in either direction.

Path taken by the impulse. — An attempt has been made to determine the path taken by the impulse started artificially in the conducting system. This question has been approached by way of a working hypothesis, based upon the view of Tawara, to the effect that the impulses of the conducting system pass directly to the region of the papillary muscles, where some terminate in the ventricular musculature, while the rest pass on subendocardially to all other regions of the ventricles.

Several facts bearing upon this subject have been mentioned above. (a) Upon the basis of the hypothesis we should expect to find, since the distance to the papillary muscles is presumably greater by way of the central (septal) end of a cut false tendon than by way of the peripheral (papillary) end, that the reaction time to stimulation of the former is longer than that resulting from stimulation of the latter. This does not seem to be the case. Possibly, however, the distances are not so different as the points of attachment of the two ends of the false tendon would lead us to believe. It may be, for instance, that the Purkinje tissue at the papillary end passes some distance down the papillary muscle before making contact with the ventricular musculature. (b) The fact that stimulation of the central end of any false tendon whatever causes a contraction of the ventricles would likewise seem to indicate that there are other paths to the general ventricular musculature than those passing by way of the papillary muscles. At any rate, it does not seem likely that fibres in such a system as is described by Tawara could pass in both directions through all paths and still reach the ventricular muscle only by way of the papillary muscles. (c) The experiments detailed above, in which the reaction time to stimulation of false tendons was compared with the reaction time to stimulation of the heart wall close to the insertion of the false tendon, might also be taken to indicate that the impulse is delivered to the ventricular musculature at, or close to, the insertion of the false tendon, were it not for the difficulties in interpretation that have been mentioned.

Ring \acute{e} ing the base of the false tendon. — The problem has been attacked in another way. It was premised that if the septal ends of the false tendons do not connect with the ventricular musculature directly at

their points of insertion into the septum, but pass to the papillary muscle, or at least to some distant point, superficial cuts, just deep enough to sever all of the subendocardial Purkinje tissue, made successively, and so placed around the point of insertion of a false tendon as eventually to ring it, should annul the response of the tendon so ringed to stimulation, or should lengthen the reaction time. For the purpose of making this cut a knife was used which could not cut deeper than 2-3 mm. Two sketches are here given (Figs. 1 and 2) to indicate the location of these cuts.

FIGURE 1.

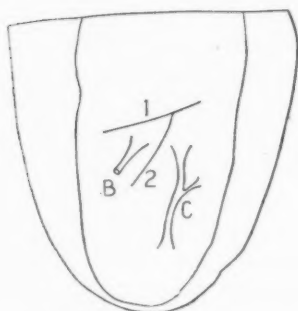


FIGURE 2.

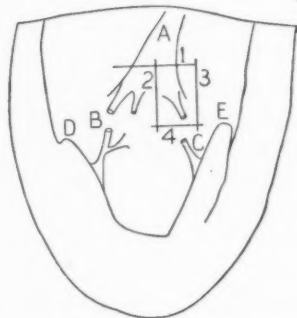


FIGURE 1. — Shows the position of the false tendon and of the cuts in Experiment No. 10.

The outer wall of the left ventricle has been removed so as to expose the left side of the interventricular septum. (B) Septal stump of the cut posterior false tendon. (C) Anterior false tendon. 1 and 2, Cuts severing the Purkinje tissue directly continuous with B.

FIGURE 2. — Shows the position of the false tendon and of the cuts in Experiment No. 11.

A part of the outer wall of the left ventricle has been removed so as to expose the left side of the interventricular septum and the papillary muscles. (A) Left branch of the bundle of His (white streak). (B) Posterior false tendon cut as indicated. (C) Anterior false tendon cut as indicated. (D) Posterior papillary muscle. (E) Anterior papillary muscle. 1, 2, 3, and 4, Cuts insulating septal stump of the anterior false tendon.

The interpretation of the results of ringing is, however, quite complicated. For instance, (a) the ringing of a tendon requires considerable time, during which the irritability of the heart as a whole may change; (b) the ringing undoubtedly affects unfavorably the circulation in the false tendons and subjacent heart muscle; (c) injury to Purkinje tissue in one part of the system may affect unfavorably its

irritability in other places; (d) the change in the direction of conduction resulting from the cuts may cause alterations in the latent period of response.

The results of the five experiments in which the ringing of a false tendon has been attempted, have been collected and arranged to suit present purposes in Table VI.

Perusal of this table shows that upon the whole the result of the successive cuts is to lengthen the latent period of ventricular response, or to increase the minimum stimulus necessary for a response, or both. More specifically the results are as follows: (a) When the first cut divides the Purkinje tissue above the insertion of the false tendon (Exps. 10, 11), the effects are either very slight or entirely lacking. (b) When the first cut divides the Purkinje tissue below the insertion of the false tendon (Exps. 19, 20), the most marked effects of any first cut are obtained. The latent period is lengthened and usually the threshold is raised. In one case (Exp. 20) the false tendon temporarily lost its irritability. (c) When the first cut is parallel to the course of the bundle and merely severs the Purkinje tissue in the septum and between the two main false tendons (Exp. 17), no noteworthy changes occur. (d) The most striking results follow the final cut made in the attempt to complete the ringing of the false tendon. Whether this final cut happens to be above or below the insertion of the false tendon, the result is practically the same.

It so happened that in the only two experiments in which it was demonstrated at autopsy that the ringing was complete (Exps. 10, 11, see Figs. 1 and 2), the completing cut was the one below. In one case the posterior, in the other the anterior false tendon was ringed. In both cases not alone the ringed (septal) end of the false tendon lost its irritability after the completing cut, but also the opposite (papillary) stump, or corresponding papillary muscle of the same false tendon. When this first happened (Exp. 10, in which the papillary muscle was directly stimulated), it was assumed that the whole heart had lost its irritability, and the experiment was consequently brought to a close. In the second instance it was found, however, that the posterior false tendon was still irritable, when, as a result of ringing the septal end of the anterior false tendon, both the septal and papillary ends of this tendon had completely lost their irritability.

In three of the cases it was found at autopsy that the base of the

TABLE VI.

No. of experiment.	False tendon.	Position of cuts.	Order of cuts.	Latent period.		Threshold position of coil.	
				Before.	After.	Before.	After.
10	Posterior	Above	1st	0.1	0.1	13	13
		Below	2d	0.1	Not irritable ¹	13	0
		Between	Not made
		Outside	
11	Anterior	Above	1st	0.8-0.1	0.1	13	13+
		Below	4th	0.125	Not irritable ³	13	0
		Between	2d	0.1	0.105	13	13
		Outside	3d	0.12 ²	0.125	13	13
17	Posterior	Above	3d ⁴	0.12-	Not irritable ⁵	11	0
		Below	2d	0.11	0.12-	14	11
		Between	1st	0.11-	0.11	11.5	14
		Outside	2d	See above	See above	See above	See above

19	Anterior	<div style="display: flex; align-items: center; justify-content: center;"> <div style="margin-right: 5px;">{</div> <div style="display: flex; flex-direction: column; align-items: center;"> <div>Above</div> <div>Below</div> <div>Between</div> <div>Outside</div> </div> <div style="margin-left: 5px;">}</div> </div>	3d ⁷	0.11 —	0.15	24.5	24.5
			1st	0.095 ⁸	0.11 +	26	25
			1st	See above	See above	See above	See above
			2d	0.11 —	0.11 —	25.5	24.5
20	Anterior	<div style="display: flex; align-items: center; justify-content: center;"> <div style="margin-right: 5px;">{</div> <div style="display: flex; flex-direction: column; align-items: center;"> <div>Above</div> <div>Below</div> <div>Between</div> <div>Outside</div> </div> <div style="margin-left: 5px;">}</div> </div>	3d	0.2	0.16 ⁸ ⁹	14.25	8
			1st	0.117	0.14 ⁸	26	17
			2d	0.156	0.2	15	14.25
			4th	0.16	0.18	8	6

¹ At this time the same (posterior) papillary muscle also lost its irritability.

² At this time the peripheral end of the same false tendon lost its irritability, while the other false tendon remained irritable, its latent period being 0.12 second.

³ Between cuts 2 and 3 the heart began to beat rapidly. This possibly accounts for the lengthened latent period at this place.

⁴ At autopsy it was found that this cut did not completely ring the tendon. It failed to intersect cut number 1, leaving intact at this point an isthmus of endocardium about $\frac{1}{2}$ mm. wide.

⁵ The posterior (same) papillary muscle was still irritable.

⁶ Following this cut the ventricles contracted at a very rapid rate; the long latent period following the cut may be the result of this.

⁷ This cut did not completely ring the tendon. At two points located at the bottom of deep grooves in the septum the knife had failed to reach the endocardium.

⁸ Immediately after this cut the false tendon lost its irritability, but regained it later.

⁹ Along the path of this cut there was left uncut an isthmus of endocardium about 1 mm. wide.

false tendon had not been completely ringed. The results of these experiments were not exactly alike, possibly because the uncut Purkinje tissue varied in amount. Nevertheless the general trend of the results bears out those of the successful cases of ringing. Thus in one of the cases (Exp. 17), while with the last cut the septal (ringed) end of the false tendon lost its irritability, the corresponding papillary muscle remained irritable; the heart fibrillated when this papillary muscle was stimulated. In another case (Exp. 19) the false tendon did not lose its irritability with the last cut, although the latent period of response of the ventricles was very greatly lengthened, a result which does not usually follow incision above the insertion of the false tendon unless, as in this case, it happens to be the final cut. In the third of these cases of incomplete ringing (Exp. 20), the false tendon temporarily lost its irritability in consequence of the third cut, which again was above the false tendon.

GENERAL DISCUSSION.

It will be seen that in certain respects these facts appear to bear out the hypothesis suggested by the work of Tawara: (1) The fact that complete ringing blocks completely the passage of the impulse into the ventricular musculature is strongly indicative of the correctness of the theory in general. It is appreciated, however, that the interpretation of this result is complicated by a number of considerations. (a) For instance, the operation for complete ringing is more extensive than for incomplete ringing. There is, however, no reason for believing that the severity of the operation was to any considerable extent greater in those instances in which the ringing was not quite complete than in those in which the ringing was complete. Yet in two of the three former cases the impulse was only partially blocked for a short while. (b) It must be borne in mind that the cut severs in addition to the subendocardial Purkinje tissue, some of the underlying heart muscle. It is possible that the impulse from the false tendon is delivered to the superficial muscle bundles, and cannot reach the mass of the musculature after the latter have been divided. That this factor is of no importance is rendered probable by some trials made on the dog's heart in which the irritability to electrical stimulation of a point on the surface of the heart was tested while it was being ringed with

the knife used for this purpose in the experiments on the calf's heart. The irritability of the area did not change as it was ringed. (c) The complete ringing might so impair the circulation in the isolated Purkinje tissue as to interfere with its irritability. That this is of little importance is indicated by the fact that the cut (namely, the cut above) that deprives the false tendon of its main blood supply has the least effect on the response of the false tendon to stimulation. Furthermore, it is scarcely conceivable that the main arteries could have escaped in all of the cases of incomplete ringing, yet the false tendon in two of these cases failed to lose their irritability. The point made below under (2) likewise indicates that the effect of ringing is not, or at least not wholly, explicable upon the basis of disturbed nutrition in the tendon.

(2) The fact that complete ringing deprives of its irritability not alone the ringed (septal) end of the false tendon, but also its opposite (papillary) end, would also seem to be in favor of the hypothesis, although we are not as yet prepared to offer any explanation of this curious fact.¹⁸

(3) Our observation that in the case of a single cut the one below the insertion of the false tendon is followed by more striking results than the one above, also supports the theory.

(4) The fact, however, that the ventricles responded to stimulation of the false tendon after cuts so placed as to leave, as the one path open to the impulse, one leading some distance upward into the main left branch of the bundle of His (Exps. 17 and 19 and also, owing to the incompleteness of the cut, 20) would seem to be inconsistent with the hypothesis unless it is assumed that the impulse carried upward again finds its way to the region of the papillary muscles.

Most of the observations, therefore, fit into the view of Tawara, while none are unalterably inconsistent with it. It should be added that

¹⁸ HERING, in one case, has noted in the dog a phenomenon possibly related to this. The right limb of the conducting system was divided above the anterior papillary muscle. For a while thereafter this papillary muscle failed to contract with the rest of the ventricles, and when it did eventually contract the interval between the contractions of the conus and the auricles was longer than while the papillary muscle was contracting before the cut. Hering believes this result fits into TAWARA's observation that the first arborizations of the auriculo-ventricular bundle occur in the vicinity of the papillary muscles. (HERING: *Archiv für die gesammte Physiologie*, 1909, cxxvi, p. 225.)

technical difficulties have stood in the way of determining what the effects of ringing the papillary end of a false tendon might be.

BEARING OF THE OBSERVATIONS ON THE CAUSE OF THE AS-Vs
PERIOD.

A discussion of the As-Vs pause in the mammalian heart in the light of present views in regard to the anatomy and physiology of the His-Tawara system may not be without interest. Since the discovery of the function of the auriculo-ventricular bundle of His, it has been generally assumed by physiologists that the As-Vs pause is the result of the slenderness of the structure, the His bundle, through which the impulse must pass on its way from the auricles to the ventricles. This assumption seemed to be justified by the observations of Gaskell and others on the delay suffered by the impulse in passing over artificially made bridges of heart muscle. Our results make it obvious that this assumption, at least in so far as it applies to the narrow strands of the false tendons, is not admissible.¹⁹ A few figures will serve to make this clear. Assuming that conduction through the rest of the conducting system is at the same rate as in false tendons (about 75 cm. per second), and assuming, furthermore, that the impulse is delivered to the musculature of the papillary muscles (in a calf's heart this, the distance travelled by the impulse, would be approximately 5 cm.) the As-Vs interval of the calf's heart, were it determined entirely by the time consumed by the impulse in traversing the conducting system, would be about 0.066 second. In our experiments the As-Vs interval has always been much longer than this, usually about 0.14 seconds. Evidently conduction similar to that in false tendons but through Purkinje tissue, cannot be the only factor contributing to the As-Vs interval.

It is of more than passing interest that the sum of the reaction time of the ventricles to direct stimulation as determined in our experiments (0.08-0.1 second) and of the time required for the transmission of the impulse from the auricles to the ventricles, as calculated above (0.066

¹⁹ We should not lose sight of the fact that so-called slowed conduction through bridges or compressed areas is always the result of injury, and of an altered distribution of the impulse to the parts beyond the injury. Although false tendons are narrow, they are not injured.

second) gives a figure (0.146-0.166) that is not far removed from the As-Vs interval (about 0.144 second) most frequently observed in this series of experiments. This coincidence suggests the question, Will an analysis of the anatomy of the conducting system serve to direct us to some arrangement, such, for example, as a synapse, muscular or nervous, that would permit of the manifestation of something of the nature of a latent period?

Our knowledge of the arrangement of the nervous connections of the auricles and ventricles has not as yet advanced to such a point as to render a discussion of it profitable in this connection. The conducting system of Tawara does, however, present several junctions of morphologically different cells. Briefly, these are found in the following places:

(1) At the union of the atrial with the funicular fibres. In certain animals, the sheep, for example, a considerable number of the atrial fibres, according to Mönckeberg, end freely in the abundant fat tissue that usually occurs about the atrial section of the auriculo-ventricular bundle. Nevertheless in this animal unions can be seen here, the fibrils of one cell passing over unbroken into cells of different type.²⁰ On the other hand, in other animals, the dog for example, it is impossible to distinguish a hard and fast line of transition here. The atrial type of cell changes gradually into the funicular type.²¹

(2) At the union of the nodal and the Purkinje type of cells. This, however, seems to occur gradually, without any sharp line of demarcation.

(3) At the union of Purkinje fibres with the ventricular heart muscle. This, likewise, is very gradual, so that it is scarcely possible to recognize a boundary between them.

Clear experimental evidence has been presented against there being any appreciable delay in the transmission of the impulse over the union of Purkinje fibres with ventricular heart muscle, in that the reaction times to stimulation of a false tendon and of the heart wall directly have practically the same durations.

There is no direct experimental evidence for or against there being a delay in the transmission of the impulse in either of the other above-mentioned locations, other than the fact, demonstrated by Hering,²²

²⁰ MÖNCKEBERG: Untersuchungen über das Atrioventrikulärbandel im menschlichen Herzen, Jena, 1908.

²¹ TAWARA: *Loc. cit.*, p. 137.

²² HERING: *Loc. cit.*

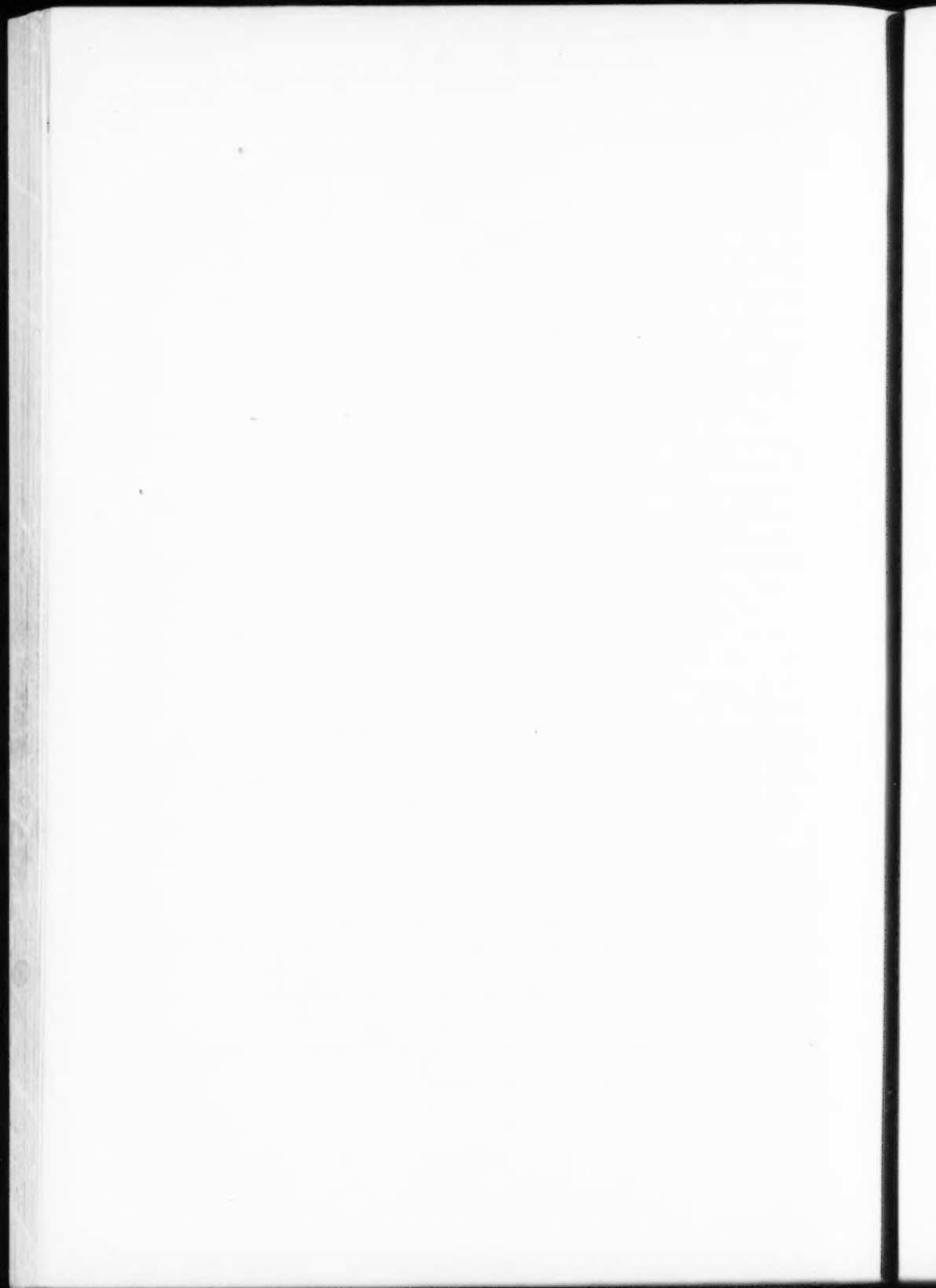
that the main delay in transmission in either direction is in the auricular portion of the auriculo-ventricular bundle. Hering assigns the delay entirely to the node of Tawara. No other possibility is considered. While there is no denying that Hering's inference may be correct, we do not believe it is possible, as yet, to exclude other factors, one of which may be a latency suffered by the impulse in traversing one of the two junctions in this locality. By no great stretch of the imagination it is possible to recognize a certain similarity of the atrio-funicular junction in the sheep to a bridge between heart masses separated by a clamp or by incisions. In both there is at this place a break in the continuity of a majority of the fibres. In view of the rapid rate of conduction that obtains in those parts of the conducting system that are open to direct experimentation, the writer can see no good reason for believing that the complexity of the fibres in the node constitutes an explanation of the delay in the transmission of the impulse from auricles to ventricles. If the knot-like structure of the node of Tawara is the cause of the delay, how is it possible to explain the rapid rate of conduction in the ventricular portion of the conducting system where "nodes" are likewise to be found.²³ Furthermore, if it is assumed that the node alone is the cause of the delay in the transmission of the impulse from auricles to ventricles, it becomes difficult to explain satisfactorily the fact that the Vs-As interval is longer than the As-Vs interval. On the other hand, explanations with some degree of plausibility can be found if it is assumed that the delay is the result of the interposition of a latent period. It is only necessary to assume then that the latent periods of the two contiguous tissues are not quite the same; or that the stimulus delivered in one direction has not the same strength as the stimulus delivered in the other.

It may be not entirely irrelevant to add that the presence of a node is not essential to the establishment of delays in the conducting system. In many of the author's experiments on heart block, the heart clamp has surrounded the auriculo-ventricular bundle some distance below the node of Tawara. Yet in these cases compression, which conceivably may act by reducing the number of connecting fibres, that is, by producing a bridge, has increased the As-Vs interval.

This discussion may be summed up with the statement that in the opinion of the writer the As-Vs interval is accounted for in relatively

²³ TAWARA: *Loc. cit.*, p. 146.

small part by the time consumed by the impulse in travelling with considerable velocity through the tissue of the conducting system. The remainder of the interval may be attributable to a latency occurring at some point of transition of one type of tissue to another. It cannot be denied that a part of this delay may occur in the node of Tawara. It is maintained, however, that the proof that such is the case is still forthcoming.



EXTRASYSTOLES IN THE MAMMALIAN HEART CAUSED BY THE STIMULATION OF THE KEITH-FLACK NODE.

By W. D. SANSUM.

[From the Physiological Laboratory of the University of Wisconsin.]

INTRODUCTION.

IN a paper published in 1907 Hirschfelder and Eyster¹ attempted to determine that portion of the mammalian heart in which the cardiac impulse arises, using the method previously employed by Engelmann² and others in the cold-blooded heart. Hirschfelder and Eyster outline their purpose as follows:

"The investigations of Engelmann upon the frog's heart have shown that in an extrasystole arising from a stimulus to the sinus region the extrasystole and its diastolic phase (compensatory pause) plus the preceding cardiac cycle is shorter than the length of the time required for two regular cardiac cycles. In the extrasystoles arising from stimulation of the auricle or ventricle, this interval is, however, just equal to two regular cardiac cycles. The explanation of this given by Engelmann led him to conclude that by this method it is possible to differentiate between that portion of the heart that initiates the prevalent rhythm and other portions."

Hirschfelder and Eyster stimulated various regions on the vena cava and the right auricle, but were unable to determine an essential difference in the length of the compensatory pause in the different regions, and hence came to no definite conclusions as to the site of the autogenic tissue. Extrasystoles from the right auricle as well as from the superior vena cava showed in many cases a shortened compensatory pause, and on the other hand stimulation of the superior vena cava under certain conditions resulted in a full compensatory pause.

Since this time, as the result of the anatomical work of Keith and Flack,³ Koch,⁴ Aschoff,⁵ and others, that portion of the mammalian heart corresponding embryologically to the sinus region of the cold-blooded heart has been definitely located. The tissue in this region is easily differentiated from the surrounding auricle, and histologically its position and extent in the dog's heart have been carefully described by Lewis, Openheimer, and Openheimer.⁶ It lies along the sulcus terminalis of the right auricle, extending from the angle formed by the superior vena cava for a distance of about 12 millimetres. The width varies from 2 to 6 millimetres. While the experimental work that has been done in an attempt to test the significance of this region in reference to the control of the cardiac rhythm has been somewhat contradictory, the majority of workers who have studied the so-called sinus node from the physiological standpoint have concluded that under normal conditions it is this tissue in which the dominant rhythm of the heart resides.

The experiments we are about to describe were undertaken to determine whether or not any essential difference could be shown in the length of the compensatory pause in an extrasystole arising from stimulation of the sinus region and of other parts of the heart. Such should be the case if it be true that this region normally acts as a pacemaker for the whole heart.

METHODS.

Dogs and cats were used. The animals were anesthetized, placed under artificial respiration, the thorax opened after ligation of the internal mammary arteries, and the pericardium slit open and sewed to the margin of the opening in the thoracic wall. The apparatus used and the method pursued were in general the same as that described by Hirschfelder and Eyster.¹ The apparatus is constructed in such a manner that stimuli may be sent to the heart automatically at every eighth beat and during any part of the heart cycle. Unipolar stimulation was used. A non-polarizable electrode was used for the stimulating electrode. The end of this carried a piece of yarn in contact with the kaolin, by means of which the electrode easily followed the motion of the heart without breaking contact. The electric stimulus used was just strong enough to be felt on the tip of the tongue.

The indifferent electrode was fastened to the leg of the animal. An electrically driven tuning fork vibrating at the rate of 100 times per second was used to record the time.

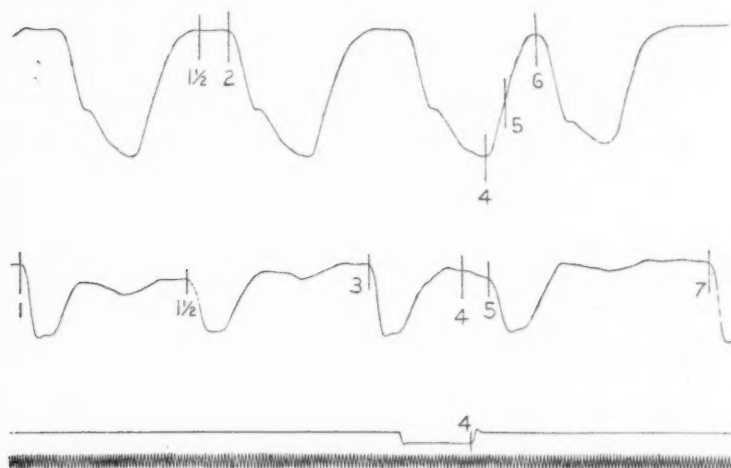


FIGURE 1.—Shortened bigemini following the stimulation of the Keith-Flack node.

Uppermost line, right ventricle; second line from the top, right auricle; third line from the top, magneto-marking pen; lowermost line, time in hundredths of a second. The occurrence of the stimulus is given by the up-stroke of the magneto-signal-pen, and is marked on the record by 4. The incidence of stimulation is the time from the tip of the previous auricular contraction to this point. The latent period of the auricles and ventricles are the times from this line to the auricular and ventricular contractions respectively. The "Double Cardiac Cycle" is the time represented by the distance between the lines 1 and 3. "Regular Systole plus Extrasystole" is represented between the lines 3 and 7, and "Extrasystolic Cycle" between the lines 5 and 7. The shortening of the bigemini is the difference between the double cardiac cycle and regular systole plus extrasystole. The normal auricular-ventricular conduction lies between the lines 1 and 2; the extrasystolic auricular-ventricular conduction between the lines 5 and 6. The same figures are used in the interpretation of all of the tracings.

RESULTS.

Table I consists of a series of results derived from the stimulation of the Keith-Flack node at different intervals during the diastolic phase. Fig. 1 shows an example from this table. In every case in this series where the stimulus is effective there is a shortening of

TABLE I.
VARYING INCIDENCE OF STIMULUS, STIMULATION OF THE KEITH-FLACK NODE.

Incidence of stimulus in diastolic phase.	Latent period.		Double cardiac cycle.	Regular systolic and extra-systolic.	Extra-systolic cycle of R. A.	Length of preceding cycle.	Inc. in length of extra-systolic cycle.	Per cent of increase in length.	Shortening of bigemini.	Per cent of shortening of length.
	R. A.	R. V.								
R. A.										
0.03-0.09(15) ¹	0.08	0.18	0.69	0.57	0.35	0.34	0.01	3	0.12	17
0.10-0.15(8)	0.06	0.15	0.75	0.65	0.40	0.38	0.02	5	0.10	13
0.16-0.20(6)	0.09	0.17	0.88	0.75	0.45	0.44	0.01	2	0.13	14
0.21-0.24(4)	0.08	0.17	1.18	0.95	0.59	0.58	0.01	1	0.23	19
0.26-0.30(5)	0.08	0.15	1.20	1.01	0.61	0.59	0.02	3	0.19	15
0.32-0.35(4)	0.07	0.16	1.22	1.06	0.62	0.61	0.01	1	0.16	13
0.36-0.38(3)	0.08	0.18	1.10	1.06	0.57	0.55	0.02	3	0.04	3

TABLE II.
STIMULATION OF AURICLE MIDWAY BETWEEN KEITH-FLACK NODE AND THE AURICULAR APPENDAGE.

	Latent period.		Double cardiac cycle.	Regular systolic and extra-systolic.	Extra-systolic cycle of R. A.	Length of preceding cycle.	Inc. in length of extra-systolic cycle.	Per cent of increase in length.	Shortening of bigemini.	Per cent of shortening of length.
	R. A.	R. V.								
0.06	0.10	0.22	0.72	0.55	0.34	0.36	none	none	0.17	23
0.07	0.09	0.21	0.68	0.58	0.36	0.34	0.02	5	0.10	14
0.10	0.10	0.21	0.88	0.79	0.53	0.44	0.09	20	0.09	10
0.11	0.05	0.15	0.69	0.61	0.38	0.35	0.03	8	0.08	11
0.11	0.07	0.14	0.67	0.63	0.38	0.33	0.05	15	0.04	6
0.12	0.11	0.21	0.89	0.80	0.52	0.44	0.08	18	0.09	10
0.13	0.05	0.14	0.70	0.60	0.40	0.35	0.05	14	0.10	14

TABLE III.
STIMULATION OF THE TIP OF THE APPENDAGE OF THE RIGHT AURICLE.

0.13	0.12	0.21	0.88	0.82	0.55	0.45	0.10	22	0.06	6
0.14	0.09	0.19	0.89	0.85	0.59	0.44	0.15	34	0.04	4
0.14	0.09	0.20	0.98	0.83	0.55	0.44	0.11	25	0.15	15
0.16	0.04	0.15	0.79	0.78	0.46	0.39	0.07	18	0.01	1
0.17	0.06	0.14	0.67	0.63	0.35	0.34	0.01	3	0.04	6
0.19-0.25(5)	0.08	0.19	0.90	0.90	0.55	0.44	0.11	25	full	full
0.27-0.35(4)	0.08	0.19	0.88	0.89	0.48	0.43	0.05	11	full	full

TABLE IV.
STIMULATION OF THE RIGHT VENTRICLE.

R. V.		0.08	0.80	0.80	0.52	0.40	0.12	30	full	full
0.08-0.12(6)	...	0.08	0.80	0.82	0.52	0.40	0.12	30	full	full
0.12-0.17(6)	...	0.08	0.82	0.84	0.52	0.42	0.10	24	full	full
0.18-0.21(6)	...	0.08	0.84	0.84	0.52	0.42	0.10	24	full	full

† To save space many of the computations have been averaged. The figures inclosed in parentheses in the first column indicate the number of such computations that have been included in each average.

the bigeminus.⁷ This result is the same as that obtained by Engelmann² on stimulation of the sinus region of the frog's heart. The eighth column of Table I shows that as a rule the extrasystolic cycle is a little longer than the normal. This is probably merely an expression of the fact that the region requires a slightly longer time to

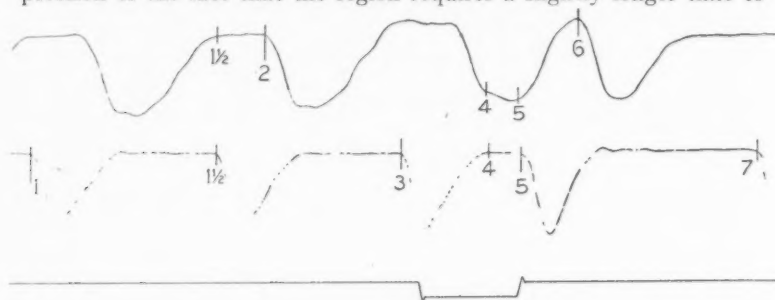


FIGURE 2.—One half the original size. Stimulation of the auricle midway between the node and the appendage. This shows the nearly full bigeminus followed by a slightly lengthened regular cycle.

regain its power to discharge an impulse, when it has previously discharged more rapidly than normal, as the result of artificial stimulation. In one case this increase in length is as high as 12 per cent of a normal cycle, but the average is much smaller (a little over 4 per cent).

Tables II and III represent a compilation of similar data from records of stimulation of the right auricle. Fig. 2 is an example taken from Table II, and Fig. 3 one from Table III. In Table II the stimuli were applied to the body of the auricle midway between the sinus node and the appendage. In Table III the auricular appendage near the tip was the area stimulated. In Table IV the right ventricle was stimulated. Fig. 4 is an example taken from this table. If we consider Table III first, it is evident that the shortening of the bigemini (columns 10 and 11) while still present is much less than in the extrasystoles arising from stimulation of the sinus region. The increase in length of the extrasystolic cycle (columns 8 and 9) is considerably greater, averaging about 20 per cent of the total normal cycle. There is evidently therefore a tendency toward the occurrence of a full bigeminus,

but not to the same extent as occurs in the case of the ventricular extrasystoles contained in Table IV, where the bigemini even in extrasystoles that occur as the result of stimuli early in diastolic phase are associated with practically full bigemini. The character of the bigemini in extrasystoles from stimulation of the body of the auricle

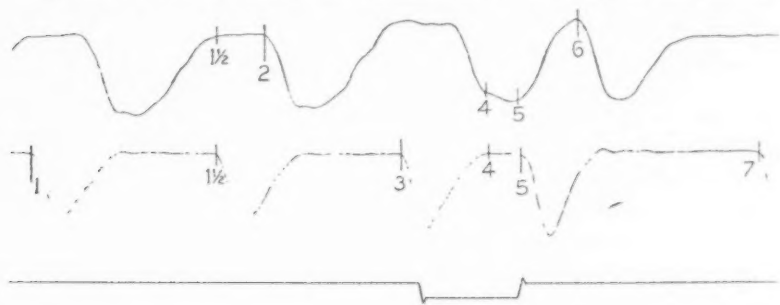


FIGURE 3.—Four fifths the original size. Stimulation of the tip of the auricular appendage. This shows the nearly full bigemini.

(Table II) on the whole are similar to those from the stimulation of the appendage. There is, however, a tendency to a greater degree of shortening and thus to approach somewhat more closely the characteristics of the sinus extrasystoles. Extrasystoles arising from the stimulation of the sinus region of the mammalian heart are therefore sharply differentiated from ventricular extrasystoles as regards the development of a compensatory pause. The condition is identical with the result obtained from the sinus and the ventricle of the heart of cold-blooded animals. The right auricle, however, as shown by Hirschfelder and Eyster¹ and confirmed in the present work, very frequently on stimulation shows the shortened bigemini, and in this differs essentially from the result obtained in the frog and tortoise. The conclusion might be drawn from this that from the functional standpoint the sinus forms in the mammalian heart merely a portion of the auricle: that portion of the auricle which possesses the greatest automaticity, but the activity of which is not separated by any interval comparable in any way to the interval that separates the activity of the auricle and ventricle in the cold-blooded heart. Against

this interpretation, however, is the distinctly greater shortening of the bigeminus in extrasystoles arising from stimulation of the sinus node than in extrasystoles from stimulation of the body or the appendage of the right auricle. This definite difference would seem to be very difficult of explanation on any such assumption. The experiments so far undertaken do not determine the cause of this difference

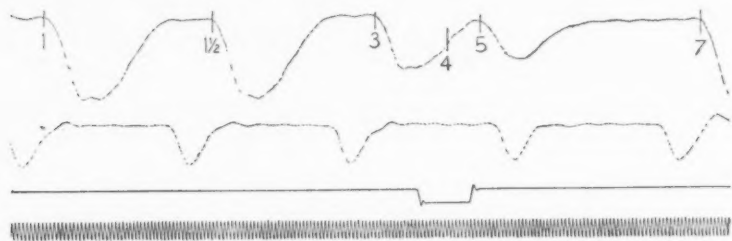


FIGURE 4. — Two thirds the original size. Stimulation of the ventricle. This shows the full compensatory pause.

with absolute certainty. A possible explanation is that the right auricle in the mammalian heart possesses such a high degree of automaticity (though less than the sinus region) that after a compensatory pause of a certain length an impulse originates in the auricle. Supposing this actually to occur, it is evident that the return to the sinus rhythm would necessitate that the next one or two auricular cycles would be longer than those before the extrasystole occurred. The result, in other words, would be a full compensatory pause, but a pause distributed over more than one cycle. Measurement of the next one or two auricular cycles following a shortened bigeminus from auricular stimulation have shown that in all cases these are somewhat longer than the auricular cycles before stimulation. Fig. 2 is an example of this. Further experimental proof must be presented, however, before this explanation can be fully accepted.

CONCLUSIONS.

Extrasystoles arising from the stimulation of the sinus region (Keith-Flack node) of the mammalian heart show a shortened bigeminus and absence of a full compensatory pause. Extrasystoles arising

ing from the stimulation of the right auricle show, in contrast to those from the cold-blooded heart, absence of a full compensatory pause. Sinus and auricular extrasystoles, however, show a distinct difference in the shortening of the pause, in the former the extrasystole is only slightly longer than a normal cycle, while in the latter the condition approaches that of the ventricle in which the lengthening is sufficient to produce a full bigeminus. It is suggested that the absence of a full bigeminus in auricular extrasystoles in the mammalian heart is due to the high automaticity of the auricle which develops to the point of activity before a full compensatory pause can be completed. These experiments, finally, add positive evidence to that accumulated by other methods that the cardiac impulse in the mammalian heart normally has its origin in the nodal tissue of the sinus region (Keith-Flack node).

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- ⁶ LEWIS, OPENHEIMER, and OPENHEIMER: *Heart*, 1911, ii, p. 147.
- ⁷ The term bigeminus was applied by Hering⁸ to designate in one word the extrasystole and the pause following it. Bigemini were "full" or "shortened," depending upon whether they were equal to or less than the length of two normal cycles.
- ⁸ HERING: *Zeitschrift für experimentelle Pathologie und Therapie*, 1906, iii, p. 511.

NORMAL TEMPERATURE OF RABBITS.

BY CHANNING FROTHINGHAM, JR., AND GEORGE R. MINOT.

[From the Laboratory of the Department of Theory and Practice of Physic,
Harvard University.]

PEMBREY,^{1,2} in a series of thirty-one observations on ten rabbits, gives the average temperature as 101.7° F. (38.7° C.) with extremes of 98.6° F. (37° C.) and 105.4° F. (40.8° C.). In a second series of seventy-seven observations on thirteen rabbits he reports the average as 103.6° F. (39.7° C.) with extremes of 101.4° F. (38.6° C.) and 105.8° F. (41° C.). Winternitz³ and Pratt find that the normal temperature of rabbits ranges from 98° F. (36.6° C.) to 102° F. (38.9° C.). Richet⁴ finds the average normal temperature to be 103.2° F. (39.55° C.), his maximum being 105.4° F. (40.8° C.) and his minimum 100.94° F. (38.3° C.). Obernier,⁵ Hale White,⁶ Ellenberger⁷ and Scheunert, Lefevre,⁸ Carter,⁹ and Abbott,¹⁰ have also made observations on the rabbit's normal temperature. The average of all the observations mentioned above is 102° F. (38.8° C.).

The variation in normal temperature ranged from 2.6° F. in Abbott's animals to 6.8° F. in Pembrey's animals. The average of the difference of extremes quoted by these writers is 4° F. The lowest normal temperature reported is 98° F. (Winternitz and Pratt) and the highest 105.8° F. (Pembrey).

Carter showed a distinct rhythm of temperature during the day with the maximum at 7-11 P. M. and the minimum some 1.2° F. lower at 7-11 A. M. Maurel¹¹ states that the rabbit's food is the chief cause of the daily variation in temperature. If the animal, having been kept without food during the day, be fed at night, the temperature shows a rise to the maximum, not at the usual time in the evening, but in the morning. This is denied by Carter, who observed an evening rise in the temperature of rabbits after a fast of three days. Abbott says: "One sees the peculiar fluctuations in temperature of over 1° C. when one feeds different amounts of food to the rabbits." These rabbits

were kept under normal conditions in clean, bright cages, and the temperature tended to be higher the larger the amount of food.

Various observations have been made upon the effect of activity on rabbits' temperatures. After making a rabbit jump for ten minutes Richet found that the temperature was lowered 0.3° F. Aronsohn and Sachs¹² found the rectal temperature of rabbits rose 1° F. after a very short chase. Richet has shown that rabbits extended on their backs and tied down lose so much heat that their temperature rapidly falls. He also thinks that the season influences the temperature with the mean in summer being 2.5° F. higher than in winter. Experiments by Obernier, Bernard,¹³ and Walther¹⁴ confirm this view. Climate has also been shown to be a factor in the variation of these animals' temperature.

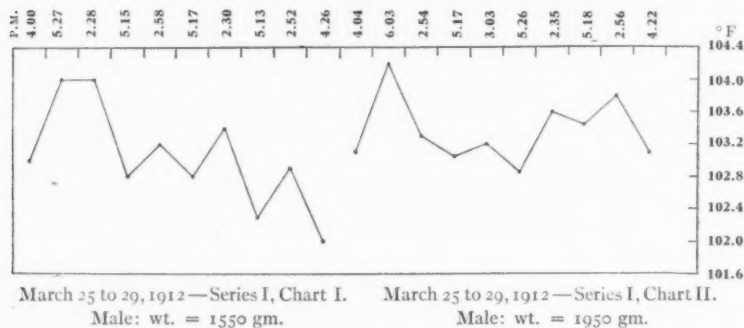
In experimental work on rabbits the temperature findings are frequently recorded, and from them conclusions are occasionally drawn, for example, the experiments of Hiss and Zinsser¹⁵ on "Leucocyte Extracts," and the experiments on the effect of drugs on rabbits' temperature mentioned by Schäfer. In other reports the temperature is considered of value, but no definite conclusions are drawn from it; such as Winternitz and Pratt's work, "On the relation of catalytic activity of the blood to the number of red corpuscles in health and to the number of white blood corpuscles and the body temperature in peritonitis."

In the laboratory of the Department of Theory and Practice in the past four years various observations have been made on the temperature of abnormal rabbits at irregular intervals. As these temperatures were variable and inconsistent, it was decided to make a short study of the temperature of normal rabbits in order to find out if possible the average variation of temperature in the animals in this department and in order to see if this variation was of such a nature that the temperature findings in experimental rabbits might be used for drawing conclusions.

Series I. — Four healthy-looking normal rabbits, three males and one female, were placed in a large pen about 12 by 4 feet and 9 feet high. This pen had a large window at one end which was partially open the greater part of the time. The rabbits stayed in this pen forty-eight hours before the temperature was recorded and remained there during the rest of the experiment. Water was kept in a basin

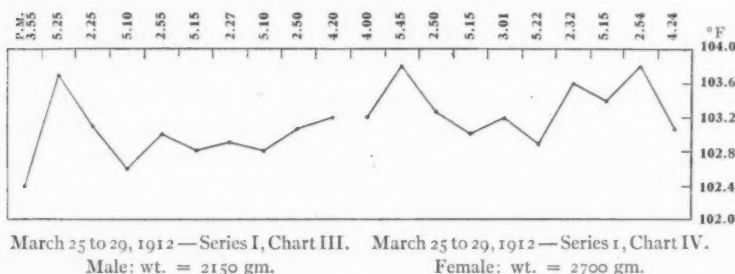
throughout the experiment, and carrots and hay were fed to them plentifully every morning and evening.

The temperatures were all taken by rectum with a standardized clinical thermometer (one-half minute) which was compared with



other thermometers and found to be the same. It was allowed to remain in the rectum at least a minute.

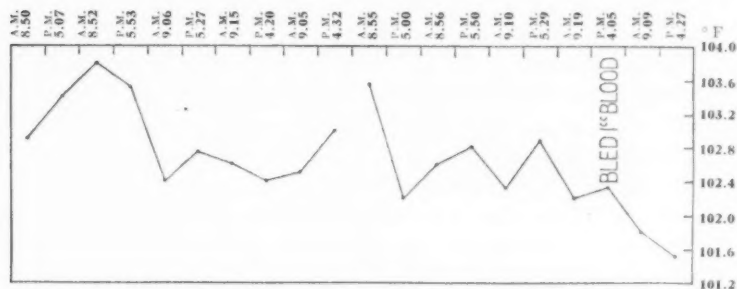
The thermometer was always inserted the same distance about 5-6 cm., for Finkler¹⁶ has shown in guinea pigs that the rectal temperature varies according to the depth to which the thermometer is



inserted. Also Abbott states "that in taking rectal temperatures you must get the thermometer in beyond the grasp of the sphincter, for you could by pressure on the bulb of this muscle force the mercury up to a point higher than that resulting from the actual body temperature."

The animals in this first series were taken from their pen in a basket as quietly and with as little handling as possible to the laboratory,

which was down two flights of stairs. The temperature was taken by holding the animal gently between one's thighs. After taking the temperatures at about 2.45 P. M. the animals were replaced in their



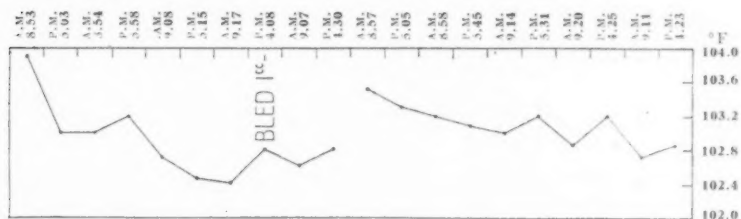
April 8 to 12, 1912—Series II, Chart I.

April 8 to 12, 1912—Series II, Chart II.

Male: wt. = 2000 gm.

Female: wt. = 2250 gm.

basket and allowed to remain without food in the laboratory for two to three hours. Then their temperatures were again recorded, and then the animals were returned to their pen. This procedure was repeated for five consecutive days, and the results are recorded on Charts Numbers 1, 2, 3, and 4, Series I.



April 8 to 12, 1912—Series II, Chart III.

April 8 to 12, 1912—Series II, Chart IV.

Female wt. = 2300 gm.

Female wt. = 2500 gm.

Series II. — In this series of four rabbits, three females, and one male, the same technic was carried out with the following exceptions. Greater efforts were made to handle and disturb the animals as little as possible. To accomplish this the temperatures of these rabbits were taken directly in the pen except for the afternoon readings for the first two days.

The temperatures in this series were taken about 9 A. M. and again

about eight hours later on five consecutive days. On the next to the last day two of these rabbits were each bled 1 c.c. One of these (No. 2) showed a drop in temperature; the other (No. 3) presented no especial change. The temperatures in this series are shown on Charts 1, 2, 3, and 4, Series II.

It is evident from these charts that the temperature of rabbits is not absolutely stable. The extremes in these animals were 101.5° F. (38.6° C.) and 104.2° F. (40.1° C.) with an average of 103.1° F. (39.9° C.) Although this average is about 1° F. higher than the average of other observers, the difference of these extremes was only 2.7° F., which is 1.3° F. less than the average of other observers. There was no regularity in the variations of the temperature. Sometimes it rose in the afternoon, sometimes it fell. No difference seemed to exist between males and females; and there was as much variation in two to three hours, Series I, as in eight hours, Series II. The greatest consistent variation between two consecutive readings was between the first and second, possibly due to the fact that the animals usually struggled more on the first day.

In conclusion it may be said that rabbit's temperature is irregularly variable. Although other observers have found variations of from 4° to 6° F. in normal rabbits, it seems from these charts that a constant variation of over 2.5° F. might be of value in drawing conclusions in experimental work.

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- ¹¹ MAUREL: Comptes rendus de la Société de Biologie, 1884, i, series 18, p. 588.

¹² ARONSOHN, E., und SACHS, J.: Archiv für die gesammte Physiologie, 1885, xxxvii, p. 232.

¹³ BERNARD, C.: Leçons sur la chaleur animale, 1876, p. 12.

¹⁴ WALTHER, A.: Archiv für Anatomie, Physiologie und wissenschaftliche Medicin, 1865, i, p. 25.

¹⁵ HISS, P. H., Jr., and ZINSSER, H.: The journal of medical research, 1908, xix, p. 323.

¹⁶ FINKLER, D.: Archiv für die gesammte Physiologie, 1882, xxix, p. 89.

STUDIES IN THE PHYSIOLOGY OF THE CENTRAL NERVOUS SYSTEM. — II. THE EFFECT OF REPEATED INJURIES TO THE SPINAL CORD DURING SPINAL SHOCK.¹

By F. H. PIKE.

[From the Department of Physiology, Columbia University.]

INTRODUCTION.

IN an earlier series of experiments on resuscitation of the central nervous system,² it was observed that transection of the spinal cord at a certain stage in the resuscitation period always resulted fatally. A cat which was breathing regularly, with pupils fairly well contracted and good reflexes, in the early period of the restoration of the head circulation, would rather suddenly begin to fail after spinal transection in the upper dorsal region. The respiration soon became slow and irregular, then ceased, the pupils dilated widely, the corneal reflex disappeared, and the heart finally became slower and weaker. Artificial respiration would keep the animal alive, so far as the heart was concerned, for a considerable period, and spontaneous respiration might return for a little time, but a second collapse would follow and the respiration would again fail. A series of such partial recoveries, each less complete than its predecessor, might occur, but the final result was permanent failure of the respiration and the reflexes about the head.

Repeated occlusions of the head arteries, with restoration of the cerebral circulation in the intervals, are well borne by cats,³ and transection of the spinal cord late in the resuscitation period has but

¹ The experiments on which this paper is based were done in the Hull Physiological Laboratory of the University of Chicago.

² STEWART *et al.*: *Journal of experimental medicine*, 1906, viii, p. 311.

³ STEWART and PIKE: *this Journal*, 1907, xix, p. 342.

slight or transient effects.⁴ In view of Sherrington's⁵ statements that shock, on transection of the spinal cord, is exerted in the aboral direction alone, and that when shock has once been produced, subsequent transections below the level of the first have only a momentary effect, this deportment of animals during the resuscitation period seemed worthy of further inquiry. The experiments given below show the result of this inquiry.

Before giving these results, it may be well to consider briefly certain of the conditions commonly known as shock and to differentiate, in a general way, some of these conditions from others with which they are sometimes confused. Porter⁶ has clearly pointed out that shock has been the occasion of much loose thinking. Yet so firmly fixed are the older ideas of shock, and so slender their real basis in fact, that any independent discussion of the subject frequently meets with heated opposition, wholly out of proportion to the evidence on which the opposition may be founded.

Porter and Story⁷ have pointed out that in certain experiments supposedly dealing with surgical shock, the medulla oblongata has been so injured anatomically as to give rise to that form of shock often described as resulting from a lesion of the central nervous system, and which I have called spinal shock. While an anatomical separation of parts, or actual anatomical injury of any part, of the central nervous system is not necessary for the production of spinal shock, complete blocking, in a fairly definite region, of conduction in all afferent or efferent paths going through that region is a necessary condition. In anaemia of the brain and medulla there is no actual anatomical lesion produced, but the effect on reflexes is exactly the same as that produced by a total transverse lesion of the spinal cord. In either case the other parts of the central nervous system remain relatively unaffected. In surgical shock no specific anatomical lesions have been described and there is probably no complete blocking of conduction paths in this condition. The area affected is very

⁴ STEWART *et al.*: *Loc. cit.*, p. 311; this Journal, 1908, xxi, p. 367.

⁵ SHERRINGTON: *Integrative action of the nervous system*, New York, 1906, p. 241.

⁶ PORTER: *Harvey Lectures*, 1906-1907, pp. 109-110, Philadelphia and London, 1908.

⁷ PORTER and STORY: this Journal, 1907, xviii, p. 196.

wide and may involve apparently the entire central nervous system. Shock, as it presents itself to the clinical neurologist, is, in general, due to a more or less definitely localized anatomical lesion and may be considered as the pathological analogue of spinal shock as it is produced experimentally. In fact Gowers⁸ has discussed it as such.

Experimental shock may be either of the nature of clinical shock or of spinal shock, and the term ceases to be an exact one. Clinical shock in itself may arise either from an organic lesion of the central nervous system, *e. g.*, a sarcoma of the vertebral column which produces a total transverse lesion of the cord, or it may arise from conditions outside of the central nervous system which produce no anatomical lesion but which in some way disturb the normal interaction between the central nervous mechanism and the periphery. Clinical shock is not, then, an exact or unequivocal term. Surgical shock usually refers to the condition brought about by changes occurring outside of the central nervous system, and influencing it only indirectly, but it may also follow an operation for removal of a tumor of the central nervous system. Traumatic shock is an equally ambiguous term since the trauma may or may not directly involve the central nervous system. Since the various conditions of shock may or may not be the same, it would be well, in discussions of the subject, to specify as accurately as possible the particular condition under discussion. Failure to observe this precaution, and a certain tendency to regard all kinds of shock as essentially alike have resulted in unnecessary bewilderment on the part of the student in this confusing and little-known field. I shall consider in this paper the condition to which the term spinal shock has been applied, meaning thereby the phenomena observed when there is total anatomical or functional interruption, at a definite site, either of a given set of, or of all, afferent and efferent tracts in the central nervous system.

When we have found out exactly what each kind of shock is, and can tell in each case what mechanisms are affected, we may discuss the question of the identity of all forms of shock with some degree of assurance. In the present state of our knowledge, to insist upon the essential identity of all these forms would appear rather arbitrary.

An animal is in a condition of spinal shock when the cerebral circulation is completely interrupted, since there is total blocking of all

⁸ GOWERS: Diseases of the nervous system, 2d ed., London, 1892, i, p. 201.

conduction paths, afferent and efferent, during the period of functional failure of the medullary and other higher mechanisms. As examples of the various views of the nature of other forms of shock, we may mention those of two workers. Romberg⁹ and his collaborators think that in collapse due to the generation of bacterial toxins in the body the synapses of the central vaso-motor mechanism are no longer passable for afferent impulses. Henderson¹⁰ believes that, in certain conditions of shock, the trouble lies in the veins and the peripheral mechanism rather than in the central mechanism. Nor is it beyond the limits of scientific probability to suggest that different forms of shock may depend not upon the disturbance of one mechanism alone, but that the two great co-ordinating mechanisms¹¹ — may be affected, each under conditions which may not greatly interfere with the essential features of the action of the other, but which may completely upset their interdependent action.

THE FUNDAMENTAL ASSUMPTIONS OF THE HYPOTHESIS OF SPINAL SHOCK.

It will be of service in the interpretation of the few experimental results given in this paper, and of others now in press or to be published shortly, to present in a general way the fundamental assumptions upon which the hypothesis of spinal shock is based, and to point out certain conclusions not sufficiently recognized as being the necessary and unavoidable deductions from these assumptions.

Without at this time going into detail concerning the earlier work and the growth of ideas of the localization of the vaso-motor nervous mechanism, we may say that present ideas of shock date from the time of publication of Goltz's¹² paper in reply to Eckhard.¹³ Eckhard had concluded, as the result of his experiments, that the mechanism

⁹ ROMBERG *et al.*: *Deutsches Archiv für klinische Medizin*, 1899, lxiv, pp. 706-712.

¹⁰ HENDERSON: *this Journal*, 1910, xxvii, p. 152.

¹¹ GASKELL: *Proceedings of the Linnæan Society of London*, Session 122, 1909-1910, p. 9 of the reprint.

¹² GOLTZ: *Archiv für die gesammte Physiologie*, 1873, vii, p. 532; *Ibid.*, 1874, viii, p. 460.

of certain vaso-motor changes involved afferent and efferent tracts passing to and from the medulla oblongata. Goltz promptly published his preliminary note and shortly afterward followed it with his longer paper on the reflexes in the dog after spinal transection.

In the interpretation of his results, Goltz, in the absence of direct proof, found it necessary to make certain assumptions. Since it is too frequently lost sight of that Goltz's ideas were based on an assumption, we may quote Goltz's own statement in the matter:—

"In brief, the whole posterior part of the animal seems unirritable (after spinal transection). A few days later the apparently dead spinal cord may have recovered almost entirely. The posterior part of the animal then offers a large number of reflex phenomena. No one will *assume* that that piece of the spinal cord which is separated from the brain in so short a time acquires entirely new powers as a reflex organ; we *must assume* that those powers were only suppressed or inhibited temporarily by the lesion of the spinal cord."¹³

Goltz's principal assumption, then, was that the reflexes seen in the part of the animal innervated by the isolated portion of the spinal cord were normally negotiated by that part of the spinal cord lying below the level of the transection. His second assumption was that the reflexes were temporarily suppressed or inhibited by the lesion of the spinal cord—the postulate or assumption of "shock." Far more attention has been devoted to a discussion of the mechanism of shock than to a discussion of the validity of the fundamental assumption to which the postulate of shock is merely subsidiary. Goltz subsequently extended his main assumption to all levels of the central nervous system below the cerebrum. In the closing sentences of his description of the deportment of his "brainless dog," he says:¹⁵ "All these facts demonstrate with great clearness that the functions [Verrichtungen] of the parts of the brain lying below the cerebral hemispheres are approximately the same in all vertebrates. For many years I have insisted that the spinal cord of the higher animals has within itself independent processes as various or manifold as has

¹³ ECKHARD: Beiträge zur Anatomie und Physiologie zu Giessen, 1873, vii, pp. 67-80, 81-113.

¹⁴ GOLTZ: translated in LOEB, Comparative physiology of the brain, New York, 1900, pp. 273-274.

¹⁵ GOLTZ: Archiv für die gesammte Physiologie, 1892, ii, p. 614.

the spinal cord of the frog. This statement may now be extended. It holds not alone for the spinal cord, but for all levels of the central nervous system lying below the cerebrum." As a necessary consequence of this main assumption, it follows, as Goltz so strongly insisted, that there are no motor centres in the cerebrum, and that the cerebrum is essentially an associative mechanism. As Loeb expressed it, the cerebral hemispheres have to do with associative memory.

Goltz's wider statement of the segmental hypothesis of the central nervous system is a strictly logical extension of his earlier statement of the functional equivalence of the spinal cord in all vertebrates, and the subsidiary assumption of shock is just as necessary in the former case as in the latter. Without the postulate of shock, Goltz's interpretation of the deportment of his brainless dog would be impossible. And granting the validity of this postulate of shock as Goltz uses it, his conclusions are perfectly sound and consistent. In fact Goltz's position has been the only consistent position with regard to the whole hypothesis.¹⁶ The fundamental assumption of the segmental hypothesis, and the secondary but inseparable assumption of shock are therefore clearly inconsistent with the hypothesis of cerebral localization.

Goltz's idea of the cause of shock was the stimulation of efferent inhibitory pathways by the act of spinal transection. Despite the arguments against the validity of such an assumption,¹⁷ the hypothesis is still widely held.

Beginning with Rosenthal¹⁸ and Bastian,¹⁹ the idea has grown up among certain groups of clinicians that the spinal cord is not the sole reflex mechanism in the human subject. This idea finds its most definite expression, perhaps, in the law of Crocq,²⁰ that the reflex tonus of the skeletal muscles and most skin reflexes in adult human subjects are dependent upon reflex arcs which pass through the cerebral cor-

¹⁶ PIKE: *Science*, 1912, N. S., xxxv, p. 621.

¹⁷ PORTER and MUHLBERG: *this Journal*, 1900, iv, p. 334; SHERRINGTON: *Integrative action of the nervous system*, New York, 1906, p. 246; PIKE: *this Journal*, 1909, xxiv, p. 124.

¹⁸ ROSENTHAL: *Sitzungsberichte der Königlichen preussischen Akademie der Wissenschaften*, Berlin, 1873, p. 104.

¹⁹ BASTIAN: *Medico-chirurgical transactions*, 1890, xxiii, p. 151.

²⁰ CROCQ: *Gazette des hopitaux*, 1901, No. 88, p. 850; Abstract in *Jahresbericht für Neurologie und Psychiatrie*, 1902, p. 82.

tex, and that the arcs for the tendon reflexes lie through the brain stem; while in new-born animals and in the lower forms generally these reflexes may depend upon short arcs through the spinal cord alone. Surprising as it may seem, the idea of phylogenetic relationships seems to be more definitely held by clinicians who for the most part deal with the higher forms alone than by the purely laboratory workers in physiology.

The present position of neurologists with reference to cerebral localization of motor functions, while considerably removed from that of Goltz, is not altogether clear on all points. The hypothesis of the functional independence of circumscribed centres, one for each conceivable function, once so firmly fixed in the minds of physiologists and still so commonly found in the literature, undoubtedly needs revision. But in such a revision the idea of localization need not necessarily lose in definiteness, but, on the contrary, may gain greatly in clearness.²¹

Von Monakow²² has shown that in ontogeny as well as in phylogeny two motor systems — the phylogenetically old and the phylogenetically new — are successively concerned in movement in man. There seems little question that cerebral localization, so far at least as the motor functions are concerned, is a fact and not a hypothesis. It seems equally clear that the phylogenetically newer mechanisms are the ones which are chiefly represented in the cerebrum and that the older mechanisms participate but little. Certain of the newer mechanisms have been added to the spinal cord also in phylogeny. A third assumption of the segmental hypothesis, implied but not expressed, is that all levels of the central nervous system below the cerebrum have remained unaffected functionally in phylogeny, since, according to Goltz's wider statement, these levels have essentially the same function in the frog, with the merest rudiments of the newer systems, and in the monkey or in man, manifesting the highest development of the newer mechanisms. Von Monakow,²³ although retaining the assumption of certain segmental mechanisms in the spinal

²¹ PIKE: *Science*, 1912, N. S., xxxv, pp. 619-622.

²² VON MONAKOW: *Aufbau und Lokalisation der Bewegung beim Menschen*; Separatabdruck aus dem Bericht über den IV Kongress für experimentelle Psychologie in Innsbruck, 1910; Leipzig, 1910.

²³ VON MONAKOW: *Loc. cit.*, Über Lokalisation der Hirnfunktionen; II allgemeinen Sitzung der Versammlung deutscher Naturforscher und Ärzte zu Königsberg, 1910; Separatabdruck, Wiesbaden, 1910.

cord, finds it necessary to renounce the greater part of the segmental hypothesis in so far as it concerns movement in man and its relation to cortical mechanisms. Shock thus becomes not only an unnecessary and confusing hypothesis in dealing with cerebral localization, but is implicitly denied in part in von Monakow's argument. It is doubtful if the hypothesis of shock is capable of adding anything to the clearness of our ideas of the function of the spinal cord. The hypothesis of a phylogenetic change, functional as well as morphological, in the central nervous system, and the assumption of integrative mechanisms²⁴ would seem to be the only necessary assumptions for the interpretation of cerebral function. It seems questionable whether any other assumptions are necessary in the interpretation of function in the spinal cord. Nor does it seem possible to neglect the strong probability of a phylogenetic change in the spinal cord in arriving at this interpretation.

The great mass of anatomical data on the nervous system available at the present time was not available when Goltz began his work. We are no longer shut up, as Goltz was, to the two assumptions open to him: (1) that the isolated part of the spinal cord acquired new functions or (2) that all the functions observed after a period of recovery were normally negotiated by the isolated portion of the spinal cord. A third assumption is now open to us — the assumption that the more primitive mechanisms may assume the functions of the newer in the event of injury to the newer. The deductions from the third assumption are supported by all the known facts of cerebral localization.

It would seem to be a truism that the proof or disproof of the various conflicting views, and the final reconciliation of such as stand the test of later work will be found in the study of the phylogenetic and ontogenetic development, functional as well as morphological, of the central nervous system; and, quite apart from any question of clinical application, such a study cannot fail to yield as one of its results a clearer insight into the rôle of the central nervous system in the evolution of the vertebrate phylum.

This is in line with the suggestion of Donaldson²⁵ that the differ-

²⁴ HERRICK: *Journal of nervous and mental disease*, 1911, xxviii, p. 730; PIKE: *Science, loc. cit.*, p. 619.

²⁵ DONALDSON: *Journal of comparative neurology and psychology*, 1908, xviii, p. 144.

ences in brain weight, spinal cord weight, and weight of the entire central nervous system in the common leopard frog, *Rana pipiens*, as compared with the corresponding European species, *Rana temporaria* and *Rana esculenta*, might be expected to correspond to certain physiological differences.

The method of attack upon the problem of the function of certain parts of the central nervous system must be to a certain extent an indirect method. One of the main problems of the present time lies in tracing out in the central nervous system the course of the nerve impulses concerned in the various activities of the organism. This is primarily a question of architecture, and while both morphologist and physiologist are concerned with essentially the same fundamental problem — the question of the course pursued by nerve impulses — the methods of attack are somewhat different. The morphologist follows out nerve tracts, but he has no assurance, from this study alone, that all impulses go in exactly the same channels which the microscopic study indicates. The anatomical possibilities should be checked up experimentally. The physiologist has as yet no direct method, capable of extensive application, of following the actual course of a nerve impulse in the central nervous system. The physiologist, along with workers in other sciences, is therefore reduced to the necessity of making certain fundamental assumptions at the start and then testing their agreement with the known facts or with new facts which these assumptions lead him to search for.

THE EFFECTS OF LOW BLOOD PRESSURE IN THEIR RELATION TO SHOCK.

The possible indirect effects, immediate or remote, of certain bodily conditions associated with spinal shock should also be considered. If the lesion is high up in the spinal cord, the blood pressure is always low. This is invariably the case when the cerebral circulation is interrupted.

The question of the effects — immediate and remote — of low blood pressure²⁶ arises then in connection with experiments on repeated injuries of the spinal cord, and on the persistence of function,

²⁶ PIKE, GUTHRIE, and STEWART: *Journal of experimental medicine*, 1908, x, p. 400; PIKE: *this Journal*, 1909, xxiv, p. 125.

which I will consider in a separate paper. Le Gallois²⁷ early in the last century found that month-old rabbits would, after beheading, show all the essential phenomena of the circulation for considerable periods of time if artificial respiration was kept up. The circulatory phenomena soon ceased if the whole spinal cord, or any large portion of it, was destroyed. Le Gallois' interpretation that the heart beat was dependent upon the presence of the spinal cord was, as Goltz²⁸ subsequently pointed out, erroneous, but the facts were good. It may be remarked in passing that Le Gallois' experiment was the forerunner of the method of decerebration of more recent years.

Goltz²⁹ found that the circulation would persist for hours in decapitated frogs. If the spinal cord was afterward destroyed in one frog and left intact in a control, the blood accumulated in the veins of the frog whose cord was destroyed and the heart soon ceased to beat, at a time when neither heart beat nor circulation was seriously affected in the control frog. In a later paper Goltz³⁰ reported that the intravascular injection of defibrinated blood would temporarily restore the heart beat in the frog whose spinal cord had been destroyed, but would not conserve it long. A fresh injection of defibrinated blood would again restore the heart beat for a comparatively short interval. Schiff³¹ found that, in dogs, an enormous volume of blood (270 to 310 gm. in dogs of seven or eight kilos) must be injected to maintain the normal blood pressure after destruction of the medulla oblongata and cervical portion of the spinal cord. Goltz³² states that his dogs died within thirty hours after total removal at one time of all that portion of the spinal cord lying below the level of transection. It is possible to remove the greater portion of the spinal cord — all below the level of the third dorsal vertebra, as Goltz and Ewald³³ have shown, by removing smaller parts in successive operations.

²⁷ LE GALLOIS: *Expériences sur le principe de la vie*, Œuvres, Paris, 1812, i, p. 34; cited by GOLTZ and SCHIFF, *vide infra*.

²⁸ GOLTZ: *Archiv für die gesammte Physiologie*, 1874, viii, p. 485.

²⁹ GOLTZ: *Archiv für pathologische Anatomie und Physiologie* (Virchow), 1864, xxix, pp. 398, 412, 415.

³⁰ GOLTZ: *Archiv für die gesammte Physiologie*, 1872, v, p. 74.

³¹ SCHIFF: *Untersuchungen zur Naturlehre des Menschen und der Thiere*, 1876, xi, p. 245; *Gesammelte Beiträge*, 1894, ii, p. 596.

³² GOLTZ: *Archiv für die gesammte Physiologie*, 1873, viii, p. 460.

³³ GOLTZ and EWALD: *Archiv für die gesammte Physiologie*, 1896, lxiii, p. 362.

Romberg and Pässler³⁴ attribute the fatal termination of many cases of acute infectious diseases, *e. g.*, diphtheria, pneumonia, to the failure of the heart and respiration as a secondary effect of the low blood pressure, rather than to a direct action of the bacterial toxins on the heart. Porter and Story³⁵ have found that if the blood pressure is lowered continuously, a critical level will be reached below which the usual reflex response of the vaso-motor centre to afferent impulses fails. This condition, once set up, is self-perpetuating rather than self-corrective.

The same effects of low blood pressure are shown in certain of the experiments cited in a previous paper³⁶ on the failure of the respiration after decerebration of the animals and subsequent spinal transection. Hayem³⁷ similarly finds in death from hæmorrhage that the respiration ceases before the heart beat fails.

THE EXPERIMENTAL RESULTS.

The technique of ligating the head arteries and the general deportment of the cat during the period of anæmia has been described in detail elsewhere.³⁸

When, as already mentioned, the spinal cord is transected during the occlusion period, without particular care to avoid hæmorrhage, irregularities soon appear in the heart rhythm and in the blood pressure curve. The heart often ceases to beat within an hour from the time the spinal transection is made.

That these results are not due to trauma or irritation of cardiac fibres alone is shown by results obtained by the intravenous injection of nine-tenths per cent sodium chloride solution at the time the transection of the spinal cord is made, or shortly afterward. Although the intravenous injection of large amounts of foreign fluid is inadvisable when the vessels are widely dilated and the heart action feeble,

³⁴ ROMBERG, PÄSSLER, BRUHNS, and MÜLLER: *Deutsches Archiv für klinische Medizin*, 1899, lxiv, p. 712; PÄSSLER and ROLLY: *Ibid.*, 1903, lxxvii, pp. 96-167.

³⁵ PORTER and STORY: *Loc. cit.*, p. 196.

³⁶ PIKE: *this Journal*, 1909, xxiv, p. 143.

³⁷ HAYEM: *Archives de physiologie normale et pathologique*, 1888, 5e serie, i, p. 103.

³⁸ STEWART and PIKE: *this Journal*, 1907, xix, p. 330; PIKE: *Ibid.*, 1909, xxiv, p. 124.

and is frequently attended by a troublesome œdema of the lungs, and other tissues,³⁹ it is possible to transect the spinal cord several times in succession without such immediately fatal results if a suitable quantity of salt solution is injected. The deportment of the animal under those conditions is shown in the protocol of

Experiment 1. (March 27, 1908.)—Adult cat. Ether. Tracheotomy. Permanent ligation of the head arteries. Artificial respiration.

- 10.45 A. M. Ligated head arteries.
- 11.33 A. M. Transected spinal cord below second dorsal segment.
- 11.34 A. M. Injected 10 c.c. 0.9 per cent NaCl solution.
- 11.37 A. M. Transected spinal cord below sixth dorsal segment.
- 11.39 A. M. Injected 10 c.c. of NaCl solution.
- 11.43 A. M. Transected cord below tenth dorsal segment. Violent movements of hind legs.
- 11.47 A. M. Injected 10 c.c. of NaCl solution.
- 11.55 A. M. Transected spinal cord below last dorsal segment. Reflexes of hind legs still active.
- 11.58 A. M. Transected spinal cord below second lumbar segment.
- 12.00 A. M. Injected 10 c.c. of NaCl solution.
- 12.16 P. M. Stopped artificial respiration.
- 12.18 P. M. Struggles. Movements of hind legs.

In this experiment it was possible to transect the spinal cord at five different levels, and certainly one could not produce much more irritation or trauma except by crushing the cord completely. At the close of the experiment, one hour and a half after ligation of the head arteries, the blood pressure was fair (45 to 60 mm. Hg) and the heart was beating strongly and regularly. The blood pressure rose after each injection of salt solution and afterward fell slowly.

If care is used and all hæmorrhage avoided as far as possible, the spinal cord may be transected during the occlusion period without materially affecting the course of the experiment or hastening the death of the animal. A protocol will make these points clear.

Experiment 2. (November 28, 1910.)—Old male cat. Much body fat.

Rupture of pleura in searching for head arteries. Failure of heart beat and respiration. Artificial respiration. Chest opened and heart

³⁹ PIKE, GUTHRIE, and STEWART: *Journal of experimental medicine*, 1908, x, pp. 400-402.

started by direct massage. Thoracic aorta clamped until respiratory gasps returned (some seven or eight minutes) after which clamp was removed. Spinal cord exposed in upper dorsal region after temporary failure of respiration but before ligation of head arteries.

12.22 P. M. Normal blood pressure. Corneal reflex had not yet returned.

12.23 P. M. Ligation of head arteries.

12.30-32 P. M. Respiratory gasps cease.

12.36 P. M. Blood pressure now falling very slowly. Spinal cord transected. Rise of blood pressure, with slow fall afterward.

12.46 P. M. Two or three kicks of hind legs, without apparent external causes. Slight transient rise in blood pressure. Left hind foot drawn up when pinched. Reflexes not especially active. Blood pressure remained very uniform, falling only about 2 mm. in twenty minutes. No irregularities of heart appeared.

1.02 P. M. Blood pressure now 25 mm. Hg; artificial respiration stopped. Very slight rise of pressure, then slow fall to base line. At

1.06 P. M. Heart beat was barely perceptible on blood pressure tracing. No struggles during asphyxia.

After transection of the spinal cord in this animal no change occurred in its condition which had not been observed in many other animals with the spinal cord anatomically intact. Other experiments have given similar results.

It is clear from the experiments quoted above and from the general effects of low blood pressures as gathered from the literature, that the fatal termination of spinal transection early in the occlusion period is not due to *trauma qua trauma* or to irritation of the spinal cord in itself. The trauma and irritation are just as great when the spinal cord is transected later in the resuscitation period, but the effects are very different.⁴⁰ At this later period they are merely transient as compared with the effects of the earlier transection.

The reason for the respiratory failure and the dilation of the pupils when the spinal transection is made early in the resuscitation period clearly lies in the low blood pressure, and in the inability of the reviving medullary mechanism to maintain blood pressure after interruption of the efferent vaso-motor paths. The spinal mechanisms are not more affected by the transection in itself at this time than at any

⁴⁰ PIKE, GUTHRIE, and STEWART: this Journal, 1908, xxi, pp. 359-371; STEWART *et al.*: *Loc. cit.*, 1906, p. 311.

other time. The damaged bulbar mechanisms are, however, more sensitive to low blood pressure and to strychnine poisoning at this time than later in the resuscitation period, just as a heart which has just been started by massage and is beginning to beat regularly is more sensitive to low blood pressure than a heart which has been beating strongly and regularly for some time. The necessity for a suitable blood pressure in the resuscitation of a damaged organ stands out with especial clearness from these facts.

None of the whole series of experiments need necessarily be considered as evidence for the contention that shock effects, in the sense of spinal shock, from spinal transection may be manifested in structures anterior to the level of the transection. It would appear also that a former statement⁴¹ concerning the centripetal propagation of shock applies to the low stage of activity accompanying low blood pressure rather than to the essential phenomena of spinal shock. These experiments show, however, that two different conditions arising from different causes associated with the same operative procedure may occur in the same animal; and that confusion of ideas inevitably arises from failure to specify the various conditions arising during an experiment. Spinal shock, so far as we have observed it, is propagated peripherally only. Other conditions known as shock may be propagated centripetally. On the hypothesis of the identity of all forms of shock, the differentiation of the two conditions is impossible.

The assumption of a more or less definitely localized bulbar vaso-motor mechanism, as opposed to a diffuse segmental mechanism, affords the simplest explanation of the observed facts. It is difficult to see how the segmental vaso-motor mechanisms of the spinal cord, once reduced to a condition of shock by the loss of the supposed tonus impulses from above or by stimulation of efferent inhibitory paths during the period of anæmia, could again be reduced to this hypothetical condition of shock either (1) by the loss of tonus impulses which were not coming down from above (as shown by the fact that the reflexes are not immediately affected by the transection of the cord early in the resuscitation period,⁴² or (2) by a second stimulation of efferent inhibitory fibres which produces no fall in blood pressure,

⁴¹ STEWART and PIKE: this Journal, 1907, xx, p. 71.

⁴² PIKE, GUTHRIE, and STEWART: this Journal, 1908, xxi, p. 367.

i. e., does not cause any more profound condition of shock in the segmental mechanisms than existed before. If we assume that the bulbar vaso-motor mechanism is the functional mechanism, the gradual rise of blood pressure during the resuscitation period is readily accounted for by the gradual recovery of function on the part of the bulbar mechanism. The collapse of the animal following transection of the spinal cord during the resuscitation period is due to the fact that the bulbar vaso-motor mechanism can no longer produce any rise in pressure by a constriction of peripheral vessels after interruption of the efferent nerve channels.

EFFECTS OF THE VAGI UPON HEART BLOCK AND VENTRICULAR RATE.

By WALTER E. GARREY.

[From the Physiological Laboratory of Washington University, St. Louis.]

INTRODUCTION.

IT is well recognized that vagus stimulation can produce heart block or increase the degree of partial block, with a consequent slowing of the ventricles. On the other hand, it has been shown by Erlanger and Hirschfelder¹ that in partial auriculo-ventricular block, produced by clamping the His bundle, stimulation of the vagi may decrease the block and cause acceleration of the ventricles. The conditions underlying these opposite results have not been sufficiently analyzed to prevent much confusion in the literature, especially the clinical literature, dealing with the action of the vagi in this condition. The present paper deals with an experimental analysis of the action of these nerves in heart block, undertaken with the idea of determining the mechanisms by which the opposite results mentioned above may be accounted for.

Our experiments show that in interpreting the results of vagus stimulation we must keep clearly in mind the fact that the vagi produce two effects which are antagonistic in character. The first of these is the depressing effect upon conductivity and strength of contraction. This effect may be wholly neutralized, so far as can be judged from the behavior of the ventricles, by the other effect which results from a coincident slowing of the rate of beat. Our experiments show, furthermore, that it is wholly unwarrantable to draw conclusions concerning the vagus innervation of the ventricles from the changes in their rate alone. This statement is based upon the fact that, when partial

¹ ERLANGER and HIRSCHFELDER: this Journal, 1906, xv, pp. 180 *et seq.*

auriculo-ventricular block exists in the turtle heart, it is possible, by vagus stimulation, to so alter the block that the rate of the ventricle may be varied in either direction, in one instance being slowed, in another accelerated.

MATERIAL AND METHOD.

Previous work² convinced the author that the heart of the turtle (*Pseudemys elegans* or *P. rugosa*) would serve the purposes of this investigation admirably for reasons which follow. The vagal inhibitory fibres do not innervate the ventricles in this animal, so that any change in ventricular rate consequent upon stimulation of the vagi must be the indirect result of the action of these nerves upon other portions of the heart. In selected individuals the left vagus lacks the chronotropic influence possessed by the right nerve, and it is thus possible to determine the action of the vagi uninfluenced by changes in rate.³ The turtle's heart also possesses the distinct advantage over the mammalian heart in that the ventricles are unable to initiate a rhythm of their own when a functionally complete block exists.

To produce the various degrees of block a clamp of the Gaskell type was used. It was provided with a micrometer-screw adjustment, and the jaws were covered with thick pure gum tubing — provisions which permitted very delicate adjustments with minimum injury to the tissues. The best results in clamping were obtained by a procedure in which the heart was exsanguinated by cutting the large veins and bleeding into the peritoneal cavity. The aortic arches were doubly ligated, cut, and turned out of the way. The clamp could then be accurately applied to any part of the heart and the desired degree of block produced by compression. Light heart levers were attached to the tissue on either side of the clamp and records of the contractions made.

EXPERIMENTAL RESULTS.

(a) *Sino-auricular block.* — In a previous article the author³ has described and published tracings which showed that it was possible, by stimulating the left vagus of the turtle, to so depress the conductivity

² GARREY, WALTER E.: this Journal, 1911, xxviii, p. 330.

³ GARREY, WALTER E.: *Loc. cit.*, (2), p. 342, Fig. 6.

and contractility of the auricles that these structures failed to beat or to conduct impulses from the beating veins of the right side to the ventricle, thus producing sino-auricular block which was functionally complete. It has since been determined that when the left vagus was unable to maintain this block it could easily be made to do so by applying a very slight degree of compression to the sino-auricular junction. Our experiments showed that this result was possible because the left vagus failed to innervate the right basal veins, which determined the cardiac rate. The only effect of the left vagi in these experiments was to produce block or to increase block produced by clamping.

When the right vagus nerve was stimulated in the above experiments, there always resulted a negative chronotropic change due to the homolateral action of the nerve on the pace-making veins, and consequently all contractions were transmitted to the auricles and ventricles. This result was due to the fact that the slowing permitted a recovery between contractions which compensated for the negative dromotropic effects, as will be shown subsequently. It has, on the other hand, been shown that it is possible to obtain sino-auricular block by right vagus stimulation;⁴ for example, prolonged, uninterrupted stimulation of the right vagus nerve with various isotonic salt solutions, notably sodium citrate and disodium phosphate, inhibited the whole heart; after some time the basal veins and sinus began to beat, but these beats, owing to the continued vagus effect upon conductivity, were blocked at the sino-auricular junction. The ultimate result was exactly comparable to the one obtained when the left vagus was stimulated.

(b) *The action of the left vagus upon auriculo-ventricular block.* — By selecting turtles in which the left vagus had no chronotropic effect it was possible to determine the exact relation of conductivity changes to the degree of auriculo-ventricular block. To test this experimentally different degrees of block were produced by compressing the auricular tissue at the auriculo-ventricular junction and then stimulating the left vagus with interrupted induction shocks of different strengths. If any effect whatever was produced it was always in the direction of increasing the degree of block. Although the auricles maintained their rate unchanged, the a/v ratio was increased and the ventricles

⁴ GARREY, WALTER E.: California State medical journal, July, 1907.

were thus either slowed or completely stopped. Even a slight vagus effect, one which was indicated on the tracings by only a slight weakening of the auricular contractions, sufficed in many instances to make a partial block functionally complete for the existing cardiac rate and thus to stop the ventricles completely (*cf.* Fig. 1).

In experiments conducted in this way there can be no question concerning the mode of action of the vagus, nor of the cause of the slowing

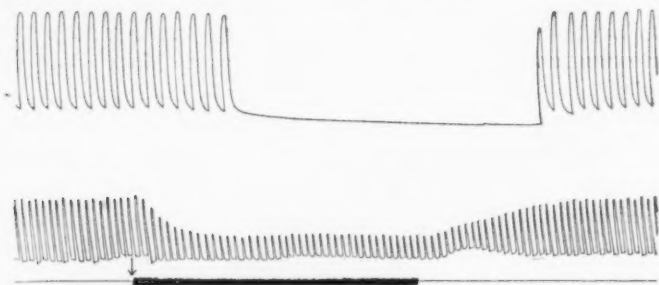


FIGURE 1.—The effect of stimulating the left vagus in partial (2/1) auriculo-ventricular block. Stimulation at ↓ depressed conductivity and weakened auricular contractions (lower tracing) without slowing their rate. The result was an increase in the degree of block with cessation of ventricular contractions (upper tracing). Initial auricular rate = 35 per minute.

or stoppage of the ventricles. The ventricles are not innervated by the vagi, and their excitability is, therefore, not decreased when the nerve is stimulated. To the contrary, the rest combined with the stimulation of augmentor fibres actually increased ventricular excitability to such a point that, when vagus stimulation ceased, evidence of the block not infrequently entirely disappeared and the normal 1/1 rhythm was re-established for a time. The vagus acted only upon the clamped area and tissue proximal to the clamp, and since the left vagus produced no chronotropic changes in the animals selected, we conclude that the increased block was due either to the weakening of the impulses or to decreased power of conduction. As a matter of fact both these factors played a rôle in the production of the results obtained. It could, however, be shown that impulses very much weaker than those pictured in Fig. 1 were able to produce ventricular contractions if the accompanying reduction of conductivity was not too great. We thus come to the conclusion that the changes in the degree of block

and in the ventricular rate, which we have described as due to left vagus stimulation, were the result chiefly of the reduction of conductivity in the clamped area. Accompanying this change a refractory state and a fatigue condition were induced in the clamped tissue by the transmission of each impulse; these factors are also important,⁵ as I have previously shown.

(c) *The action of the right vagus upon auriculo-ventricular block.*—In addition to the effects produced by the left vagus, stimulation of the right vagus nerve produces a chronotropic change the result of which is the production of effects exactly the reverse of those considered in the last section. Gaskell showed that cooling the turtle's heart, thus slowing its rate, tended to remove the condition of partial block produced by compression, while warming or irrigating with salt (sodium chloride) solution, thereby accelerating the rate, had the opposite effect. Erlanger and Hirschfelder⁶ obtained similar results in the partial blocks produced in mammals by clamping His' bundle. From these considerations it is clear we should be able to obtain evidence of this effect on the turtle's heart by stimulating the right vagus nerve and thus slowing the rate of heart beat. We should, however, expect the effects of a negative chronotropic change, and the resulting decrease in the degree of block, to be masked to a certain extent by the opposite effect due to reduced conductivity. *A priori*, we should be able, by stimulating the right vagus nerve, to produce three distinct variations in the degree of block: (1) to increase the degree of block in spite of chronotropic changes, a result in which negative dromotropic changes preponderate, (2) to produce all the usual vagus inhibitory effects without any change in the a/v ratio, in which case the antagonistic effects are just balanced, and (3) to note even marked negative inotropic and dromotropic changes upon the auricles and yet, owing to the slow rate, to see the degree of block actually decrease. Our experiments have demonstrated all these results. It is only necessary for the purposes of this paper to prove the third of these possibilities, for the greater effect necessarily involves the lesser.

In the experiment which is illustrated by Fig. 2, functionally complete block was produced by clamping the auricular tissue at the auriculo-ventricular groove. When the right vagus was stimulated

⁵ GARREY, WALTER E.: this Journal, 1912, xxx, p. 295.

⁶ ERLANGER and HIRSCHFELDER: *Loc. cit.* (1).

by weak faradic shocks (at *A*), the auricular contractions were weakened. The block remained functionally complete and the ventricles quiescent except for the single contraction following the lengthened interval between auricular contractions noted at the point marked with the arrow. Stronger stimulation of the right vagus (at *B*) caused slowing of the auricles and resulted in a complete disappearance of the a/v block. A contraction of the ventricles followed every auricular contraction. With the return of the more rapid auricular rate, complete

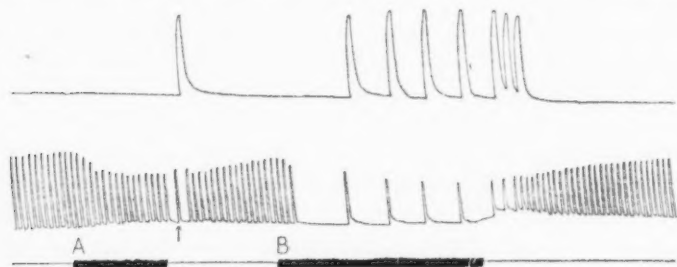


FIGURE 2. — The effect of stimulation of the right vagus nerve upon functionally complete auriculo-ventricular heart block. Upper tracing records ventricular contractions, lower tracing from the auricles. Weak faradization of the right vagus nerve beginning at *A* affected the degree of block only at ↑ when a lengthened period between auricular contractions caused a single ventricular contraction. At *B* stronger faradization of the same nerve produced marked slowing of the auricles, in consequence of which the block disappeared and the ventricles contracted after each auricular contraction. Block reappeared when the rate again became rapid. Initial auricular rate = 36 per minute.

block was again established in spite of the increasing strength of auricular contractions. It is thus seen that if the conducting system is not actually destroyed by compression the term "complete block" has a meaning which is only relative, for the degree of block depends largely upon the auricular rate. The vagi are looked upon as nerves which depress cardiac activity. It is a striking thing, therefore, to find that the negative chronotropic element in this depression, acting upon the sinus and auricles, may overbalance the other effects of depression and produce exactly the opposite effect upon the blocked ventricles. Ventricles slowed by severe partial a/v block may thus be made to beat faster; and quiescent ventricles, in functionally complete block, may be made to respond to every auricular contraction. This result is only obtainable when the auricles are slowed sufficiently

to permit a degree of recovery between contractions which more than neutralizes the negative inotropic and dromotropic changes induced by the stimulation of the vagi. The fatigue effects of the previous contractions must also be neutralized by this slowing. The fact already discussed, that stimulation of the left vagus when unaccompanied by chronotropic changes always increases the degree of block, serves as a sufficient control to prove the correctness of our contention.

It might be argued that we were stimulating sympathetic accelerator fibres along with the inhibitory. To eliminate these as factors in our results, the following procedure was resorted to, namely, stimulation of the right vagus nerve or vagus centre within the skull after destruction of the cervical portion of the spinal cord. In such cases the same results were obtained, namely, a decrease in the degree of block only when there also resulted a distinct slowing of auricular rate. The accelerator fibres of course were not affected by such intracranial stimulation.

(d) *Auriculo-auricular block, and the effects of the vagi upon it.*— In the production of auriculo-ventricular block in our experiments the clamp in reality grasped auricular tissue at the a/v junction. We know that this tissue is directly under the control of the vagi. It should be possible, therefore, to duplicate the above findings in cases of auriculo-auricular block produced by application of the clamp between any two parts of the auricular portion of the heart. This was demonstrated experimentally by removing the ventricles and so applying the clamp to the heart *in situ* that the left auricle was separated from the rest of the heart by the clamp. Compression thus produced auriculo-auricular block. Tracings from a number of turtles' hearts all showed results identical with those obtained upon a/v block. Stimulation of the ~~left~~ vagus, without chronotropic changes, always produced an increase in the degree of block, even to complete quiescence of the distal (left) auricle. Stimulation of the right vagus likewise produced an increase in the block when the negative chronotropic changes were only slight. With greater slowing of the right auricle, consequent with stimulation of the right vagus nerve proximal to the clamp, the opposite effect was produced; the degree of block became less, a larger proportion of the contractions appeared in the distal (left) auricle, which thus actually beat more rapidly than before vagus stimulation. Since the negative chrono-

tropic changes usually appeared when very weak stimuli affected the right vagus, it frequently happened that the removal of the block was the only effect of stimulation of this nerve. These experiments only serve to emphasize the fact that we are dealing with two antagonistic factors whenever the vagi are stimulated, and that block may be either increased or decreased by these nerves.

(e) *The action of the vagi upon intraventricular block.* — In the preceding experiments the tissue clamped was always a portion of the heart which was directly innervated by the inhibitory fibres of the vagus. It is generally conceded that the ventricles of the turtle do not receive such vagus fibres — a view which is verified by the experiments to be described in this section of our report. Since all vagus effects upon these structures must be indirect, it was decided to test the action of these nerves upon a block produced by clamping the ventricular musculature. The procedure employed was to section on either side of the ventricles between the middle and basal third, thus narrowing the conducting bridge so that it could be easily grasped by the clamp and any desired degree of block produced. The impulses which passed through the clamped region were initiated in the basal portion of the ventricular tissue, and with a given cardiac rate remained of constant strength, since the vagi did not depress the conductivity of this tissue. Only when a change in rate supervened, or when augmentor fibres were stimulated, did the strength of these impulses vary.

Our experiments showed that in no instance was it possible by stimulation of either vago-sympathetic trunk to increase the degree of intraventricular block; on the contrary, block in this region was always reduced when either of these nerves was stimulated in the neck (Fig. 3). This effect was invariably obtained whether there was any change in rate or not. Our experiments were then directed to an analysis of these results and showed that there were two factors involved. One of these was the stimulation of the augmentor fibres in the mixed trunk of the vago-sympathetic, the other was the reduction in cardiac rate. The action of the augmentors was demonstrated in the following way. Animals were selected in which the left vagus produced no chronotropic changes. The cerebral hemispheres were destroyed and the upper cervical cord transected. The left nerve was stimulated intracranially, intraventricular partial block having been previously

obtained by clamping. The usual auricular weakening ensued when the nerve was stimulated, but the degree of intraventricular block remained totally unaffected. Such experiments afford conclusive evidence that the depressing effects of the vagus inhibitory fibres do not extend to the clamped ventricular tissue of the turtle's heart; otherwise results similar to Fig. 1 would have been obtained, namely, an increase in the degree of block. In contrast to the absence of any effect upon v/v block obtained by intracranial stimulation, stimulation of the vago-sympathetic trunk in the neck always decreased the degree of block with consequent acceleration of the apical portion of the ventricles, distal to the clamp. This result is shown in Fig. 3.

Stimulation of the right vago-sympathetic trunk also invariably decreased the degree of partial v/v block. In the event of chronotropic changes accompanying stimulation of the right nerve it was impossible to decide between effects which were due to accelerator fibres and those indirectly due to the slower rate. Intracranial stimulation of the right vagus eliminated the accelerators of this side from consideration. The results of such intracranial stimulation are illustrated by the following experiment. Faradization of the nerve was begun with very weak shocks, the secondary coil of the (Harvard Apparatus Co.) inductorium having been withdrawn as far as possible from the primary coil and tilted to an angle of 30° . The stimuli were then gradually and continuously strengthened by decreasing the angle; thus gradual and progressive slowing of the rate was induced, as was shown upon the auricular portion of the tracings taken. At first the

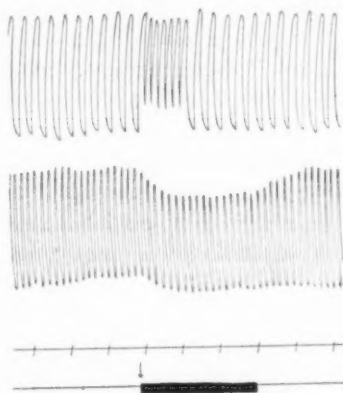


FIGURE 3. — Record of a 2/1 ventriculo-ventricular block produced by clamping across the middle portion of the ventricle; the upper tracing is from the apical portion. The left vagus trunk well down in the neck was stimulated weakly at ↓. The ensuing decrease in the degree of block was due to the action of accelerator fibres, for previous strong stimulation of inhibitory fibres of this nerve within the cranium had no effect upon the degree of block. The time record marks intervals of 10 seconds.

stimulation did not affect the degree of block, but as soon as the rate of the auricles was sufficiently reduced the degree of block decreased and the rate of the portion of the ventricle distal to the clamp increased; the ratio became 1/1. When the original heart rate returned, the original degree of block also returned. Inasmuch as our previous experiments have shown that no vagus inhibitory fibres affect the ventricle of the turtle and since intracranial stimulation of this nerve does not affect accelerator fibres, it is clear that the only variable in this type of experiment lies in the change of rate effected by the vagus stimulation. The vagus effect upon the intraventricular block is therefore wholly an indirect one; sufficient slowing of the heart will always result in a decrease in the degree of block and consequently in an acceleration of the blocked-off apical portion (unless of course the whole heart is too greatly slowed to admit of acceleration).

DISCUSSION OF RESULTS.

The main conclusions have been stated in the presentation of our experimental results, it remains only to emphasize certain phases of our work which may aid further in the interpretation and analysis of the conflicting results which may appear when the vagi are stimulated in conditions of functional heart block. Our experiments upon intraventricular block show that we must differentiate clearly between the effects produced upon a block located in a tissue which is not directly supplied by inhibitory fibres and effects produced upon a tissue subject to their depressing action. In the former case it is impossible by vagus stimulation to increase the degree of block; on the contrary, the result will almost invariably be to decrease the degree of block, because the slowing materially raises the excitability of the tissues and renders conduction more easy; in addition, the direct influence of augmentor (accelerator) fibres also adds a very material factor working in the same direction. Failure of vagus stimulation to increase the degree of block does not, however, necessarily mean that the blocking clamp (or lesion) is in a position outside of direct vagus influence; it may equally well mean that the direct depressing action of the inhibitory fibres upon conductivity is more than compensated by the action of accelerators or by such indirect and antagonistic factors as result from the slowing of the heart's rate. When vagus dromotropic and inotropic influences predominate, and the neutralizing

influences are not sufficiently potent, it is to be expected that the degree of block will be increased. Our results upon the turtle's heart are in perfect harmony with those of Erlanger and of Erlanger and Hirschfelder obtained upon mammals in which the vagi were stimulated after heart block had been produced by clamping the His bundle. These authors never noticed an increase in the degree of heart block, but obtained the opposite result (ventricular acceleration), a result which was correctly ascribed by them to the slowing of the heart's rate. Interpreted on the basis of our work, their experiments also show that if the vagi directly affected the site of the lesion, the depression produced by their stimulation was not so profound but that it was readily minimized by other factors. More recently Frédéricq⁷ has demonstrated upon mammals a finding which was previously reported by Garrey⁸ for the turtle's heart, namely, that it is possible by compression to block normal motor impulses in the heart and still show that inhibitory nerve impulses are able to pass the clamp. By this method Frédéricq demonstrated distinct vagus effects upon the dog's ventricle. It seems probable in the light of our work that discrepancies in the reports of the action of the vagi in mammalian heart block may be entirely cleared up if it is realized that the position of the blocking clamp (or lesion) is a very important consideration. Frédéricq's clamp was applied higher than Erlanger's and even grasped auricular tissue which is profoundly influenced by inhibitory fibres.

It may be well to again emphasize the fact that we are not justified from changes in rate of the ventricles, when the vagi are stimulated, to assume that the vagi innervate these structures, for if partial a/v block exists, an influence of these nerves on tissue proximal to, and even remote from, the blocking lesion may vary the ventricular rate in either direction. In any given case the result will depend upon the degree of block, the strength of vagus stimulation, the amount of slowing of the auricles, and the site of the block, that is, whether in a position slightly or profoundly under the influence of vagus inhibitory action. The ultimate result will be the algebraic sum of these varied influences and may be acceleration as frequently as slowing. It is furthermore unwarranted when such acceleration of the ventricles takes place to speak of a "reversed" action of the vagi, we must first

⁷ FRÉDÉRICQ, L.: Archives internationales de physiologie, 1912, xi, p. 405.

⁸ GARREY, WALTER E.: this Journal, 1911, xxviii, p. 249.

know that no partial block exists and then make sure that the result is not due to the ordinary negative chronotropic effects of these nerves upon the sinus and auricular regions. The pulse and apex beat alone do not afford sufficient data for the determination of the *modus operandi* of the vagi.

For the practical therapeutics of heart block our experimental analyses are also significant, for they indicate the complexity of the factors which must be considered and balanced before we can be certain that the administration of a given drug will produce the desired effect upon the degree of block.

SUMMARY.

In functional heart block, stimulation of the vagi may produce results which are exactly opposite in character. Increase in the degree of block with slowing of the rate of the portion of the heart distal to the clamp ensued whenever the tissue clamped was directly innervated by the vagi and the depressing effects of these nerves was not neutralized by antagonistic factors. Among these antagonists the action of the augmentor (accelerator) fibres was demonstrated, but of more importance was the negative chronotropic effect of the vagi. If a sufficient slowing of the rate occurred the degree of block decreased and the portion of the heart distal to the clamp was accelerated instead of slowed; or, if quiescent as a result of functionally complete block, this portion of the heart was caused to beat. The results must be ascribed to the inhibitory action of the vagi upon the cardiac pacemaker and not to accelerator action or "reversed" action of these nerves. In case the clamp was applied to a tissue not directly innervated by the vagi (the turtle's ventricles, for example) stimulation of these nerves, if any effect was produced, always decreased the degree of block. From these facts it develops that a change in the ventricular rate is not a safe criterion on which to base conclusions concerning vagus terminations in these structures. The actual effect of vagus stimulation is the resultant of several factors, among which are the position of clamp (that is, whether on tissue innervated by inhibitory fibres or not), the degree of block, the strength of vagus stimulation, the involvement of accelerator fibres in the stimulation, and the amount of chronotropic change. The experimental results were obtained on turtles' hearts, and the analysis was made possible by the differences in the action of the two vagi in the animals selected.

THE RELATIVE TOXICITY OF METHYL AND ETHYL
ALCOHOLS AS DETERMINED BY THE RATE OF
REPRODUCTION IN HYDATINA SENTA.

BY DAVID D. WHITNEY.

[From the Biological Laboratory of Wesleyan University, Middletown, Connecticut.]

DURING the past few years several investigators have been experimenting with the different alcohols in order to determine their relative toxicity. Vernon¹ in a recent paper has reviewed and published their results together with his own. Taking the toxicity of ethyl alcohol as unity, these results on various living organisms used in their experiments show that in most cases methyl alcohol is less toxic than ethyl alcohol.

While working with the rotifer, *Hydatina senta*, in connection with other problems, some experiments have been performed with methyl and ethyl alcohols (Kahlbaum), the results and conclusions of which it seems wise to record.

These rotifers were kept in watch glasses and fed upon protozoa that grew in culture media of horse manure and water. The culture water with the food into which the rotifers were placed was made up with 1 per cent of the alcohols. These animals reproduce parthenogenetically very rapidly, and as a result it is possible to obtain many generations in a short time. In these experiments a very vigorous race or family was started from a wild race, and then it was divided into three parallel families by taking three sisters and starting a family from each sister. One family was kept in normal culture water as a control, the second family was placed in 1 per cent methyl alcohol, and the third family was placed in 1 per cent ethyl alcohol. Under these different conditions the three families were kept for several generations, but at every generation the rotifers were transferred to culture water, with or

¹ VERNON: this Journal, 1911, xliii, pp. 325-342.

without the alcohols, that had been freshly prepared. As a criterion by which to determine the relative toxicity of the two alcohols, the rate of reproduction, or the number of offspring that a female could produce in a certain period of time, was selected. In order to produce an egg, which hatched shortly into a female offspring, an adult female must have been able to digest and to absorb a certain amount of food materials. If any female produced more offspring in a certain period of time than any other female, she must have been able to digest and to absorb food more quickly and also to have consumed a larger amount. Probably coupled with the metabolism were the other cellular activities of the body, as growth and elimination.

The following table gives the summaries of the results obtained by the use of the two alcohols in three different experiments:

TABLE I.

Summary of the results of the experiments showing that more offspring were produced under the influence of methyl alcohol than under the influence of ethyl alcohol; and also that the ill effects of methyl alcohol had disappeared in the second generation after it had been removed.

Generation	Control.			Methyl 1 per cent.			Ethyl 1 per cent.		
	Young females isolated.	Offspring produced.	Av. number of offspring.	Young females isolated.	Offspring produced.	Av. number of offspring.	Young females isolated.	Offspring produced.	Av. number of offspring.
6-15	118	1030	8.72	114	517	4.53	107	310	2.89
1	9	91	10.11	16	90	5.62	Experiments with methyl alcohol placed in culture water free from methyl alcohol.		
2	12	86	7.16	12	91	7.58			

This table shows that the families subjected to the influence of ethyl alcohol produced fewer offspring than the families that were subjected to the influence of methyl alcohol. These results are in agreement with those of most of the investigators in Vernon's paper which show that ethyl alcohol is more toxic than methyl alcohol.

The last portion of the table shows that no permanent injury had been done to these families which were subjected to the methyl alcohol treatment. After two families had been living for ten and fifteen generations respectively in the methyl alcohol they each produced as

many offspring as the control family when they were removed from the alcoholic solution. The effects of ethyl alcohol upon a race has been determined in an earlier paper.²

SUMMARY.

1. Methyl alcohol is less toxic than ethyl alcohol, as determined by the rate of reproduction in the rotifer, *Hydatina senta*.
2. Two families that were subjected to 1 per cent methyl alcohol during fifteen and ten generations respectively recovered from its influence in the second generation after the alcohol had been removed, thus showing that methyl alcohol produced no permanent injury to the families.

² WHITNEY: American naturalist, 1912, xlv, pp. 41-56.